FOLLICULAR GROWTH AND SERUM ESTRADIOL CONCENTRATION AS PREDICTORS OF IN VITRO FERTILIZATION OUTCOME

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FOLLICULAR GROWTH AND SERUM ESTRADIOL CONCENTRATION AS PREDICTORS OF *IN VITRO* FERTILIZATION OUTCOME

A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

by

Nicole Hausman

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I. ABSTRACT

FOLLICULAR GROWTH AND SERUM ESTRADIOL CONCENTRATION AS PREDICTORS OF *IN VITRO* FERTILIZATION OUTCOME. Nicole Hausman, Antoni J. Duleba, and David L. Olive, Section of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, Connecticut.

The outcome of *in vitro* fertilization and embryo transfer (IVF-ET) is influenced by multiple factors. Studies performed in populations with unspecified etiologies of infertility have suggested that the number of oocytes retrieved during a cycle of IVF may inversely correlate with subsequent fertilization and chance of pregnancy. The purpose of this study was to examine the relationships between pretransfer parameters in IVF-ET and the subsequent occurrence of pregnancy in patients assigned to individual diagnostic groups, with particular focus on patients with tubal disease.

The study evaluated 466 stimulated cycles which proceeded to oocyte retrievals. In patients with tubal disease, the number of follicles measuring at least 12 mm on the day of human chorionic gonadotropin (hCG) administration correlated negatively with pregnancy rates: pregnancy rates were 28.3% for those with ≤ 7 follicles and 8.7% for those with > 7 follicles ($P = 0.014$). Comparable findings were observed when correlating pregnancy rates with estradiol level on the day of hCG administration: pregnancy rates were 28.9% for those with estradiol ≤ 1,000 pg/ml and 13.3% for those with estradiol > 1,000 pg/ml ($P = 0.049$). In contrast, the opposite trends were noted in patients with polycystic ovary syndrome and unexplained infertility, with

increasing pregnancy rates at both higher numbers of follicles and higher levels of estradiol.

These findings demonstrate that ovarian response is a predictor of the success of IVF-ET in tubal disease patients, and that ovulation induction protocols may need to be individualized for each diagnostic group.

II. INTRODUCTION

A. Infertility: Nature and Magnitude of the Problem

Considering the complexity of the reproductive process, it is remarkable that 80% of couples achieve conception within one year of attempting pregnancy. Of the population attempting to conceive, a spectrum of fertility exists, extending from highly fertile couples to those with relative infertility. Infertility is usually defined as the inability of a couple practicing frequent intercourse without contraception to conceive a child within one year. It affects 15 to 20% of couples or approximately seven million people of reproductive age in the United States,[1] and may be due to multiple etiologies in one or both partners. In fact, approximately 20% of infertile couples have more than one major cause (male factor, ovulatory dysfunction, or tubal-peritoneal disease) for their inability to conceive. Without therapy, spontaneous intrauterine pregnancies do occur in infertile couples, albeit at a substantially decreased rate; thus, treatments are pragmatically aimed at shortening the time to and increasing the likelihood of conception. With a thorough evaluation of the infertile couple and application of current treatments short of *in vitro* fertilization (IVF), 50 to 60% can be expected to conceive.[2] For infertile couples experiencing failure of other available treatments, IVF with embryo transfer (ET) remains the last resort, with approximately 20% of those couples undergoing the procedure ultimately establishing a pregnancy.

B. Diagnostic Considerations

The precise incidence of the etiologic factors involved in infertility varies with the population studied. As an approximation, 15 to 20% of infertility cases are due to ovulatory dysfunction, 30 to 40% are due to pelvic

factors (endometriosis, adhesions, or tubal disease), 30 to 40% are due to male factors (abnormal semen parameters), 5 to 10% are due to abnormal sperm-cervical mucous penetration or antisperm antibodies, and 10 to 15% are due to unexplained infertility, for which no cause can be elucidated using currently available tests.[2]

1. Tubal Factor Infertility

During the past decade, the incidence of infertility caused by damage to the oviduct has increased, most probably due to an increased incidence of salpingitis. Obstruction of the oviduct may occur at either the proximal or distal portion, and may occasionally involve both regions simultaneously. The extent and location of the intrinsic and extrinsic tubal disease should be accurately determined by both hysterosalpingography and laparoscopy. The prognosis for conception after surgical tubal reconstruction depends heavily on the severity of tubal damage, with very low intrauterine pregnancy rates demonstrated in women with extensive pre-surgical obstruction. In these women, *in vitro* fertilization provides the most efficacious intervention.[3]

In the case of distal tubal obstruction, the hysterosalpingogram (HSG) aids in determining whether the tubal obstruction is complete or partial, the diameter of the hydrosalpinx (accumulation of serous fluid in the fallopian tube), and the appearance of the rugae. Beyond this, laparoscopy is usually necessary to determine the actual size of the hydrosalpinx, the thickness of the oviduct wall after distension with dye, and the presence or extent of pelvic adhesions. As previously stated, the prognosis for conception following distal tubal reconstruction correlates strongly with the extent of tubal damage present before surgery. If the fimbriae appear anatomically normal, but phimotic with only partial occlusion by adhesions, removal of

the adhesions by a fimbrioplasty procedure results in post-surgical conception rates on the order of 60%.[3] If the distal end is completely occluded and a salpingostomy (the creation of an artificial opening in a fallopian tube closed by inflammation) is necessary, conception rates following reconstruction drop to 30% with approximately 25% of these being tubal pregnancies.[4] As such, it is only logical that a positive correlation exists between the degree of distal tubal damage prior to reconstructive surgery and the incidence of ectopic pregnancies following surgery.[3]

If no dye reaches the oviduct during performance of an HSG, the diagnosis of proximal tubal obstruction must be considered. Proximal tubal obstruction is most commonly due to damage resulting from infection, but may occasionally be caused by endometriosis. Spasm of the utero-tubal junction may occur during the HSG and should also be considered in the differential diagnosis. Microsurgical tubocornual reanastomosis in women with proximal tubal damage has resulted in term pregnancy rates of approximately 50% and ectopic rates less than 10%.[3]

As a rule, if conception does not occur within 6 to 12 months of surgical reconstruction and a repeat HSG reveals recurrent tubal obstruction, a second surgical procedure is not undertaken, due to dismal follow-up pregnancy rates. These patients are subsequently referred for *in vitro* fertilization.[3]

2. Endometriosis

Although endometriosis is a benign disease, it often becomes progressive and chronic, with local infiltration and wide dissemination. By definition, endometriosis is the presence and growth of the glands and stroma of the uterine lining in an aberrant location. Although the age-

specific incidence or prevalence of endometriosis can only be approximated, it is believed that the prevalence of the disease has been increasing over the past 25 years. Estimates reveal that endometriosis is present in 5 to 15% of laparotomies performed on women of reproductive age, while the prevalence increases to 30 to 45% in those who are infertile. Heterotopic endometrial tissue grows under the cyclic influence of ovarian hormones, thus explaining onset and symptoms during the reproductive years. Most commonly, patients with endometriosis are women in their mid-30's, nulliparous, involuntarily infertile, with symptoms of dysmenorrhea and pelvic pain. The etiology of endometriosis, while not accurately elucidated, has been hypothesized to involve many factors, including retrograde menstruation, vascular dissemination, metaplasia, genetic predisposition, immunologic defects, and hormonal influences. No single theory adequately explains the many manifestations of the disease.[5]

The underlying pathophysiology producing infertility in women with endometriosis remains largely unknown, although a relationship between the severity of the disease and infertility has been established. Olive et al. reported that approximately 65% of women with mild endometriosis conceived without treatment, while only 25% and 0% of women with moderate and severe endometriosis, respectively, became pregnant with expectant management alone.[6] In the presence of moderate or severe disease with extensive adhesions involving the oviducts, the causal relationship between endometriosis and infertility seems clear. However, in patients with minimal or mild disease, a causal relationship between infertility and endometriosis has not yet been established.[3]

3. Polycystic Ovary Syndrome

The most common cause of chronic anovulation is the polycystic ovary syndrome (PCO), a complex endocrine disorder consisting of hyperandrogenism and anovulation, involving dysfunction of the hypothalamus, pituitary, ovaries, adrenals, and peripheral adipose tissues. One of the hallmarks of PCO is the relative elevation of luteinizing hormone (LH) over follicle-stimulating hormone (FSH), with exaggerated pulse levels of LH in association with low normal levels of FSH. Explanations for the relative disparity of LH to FSH have invoked hypothalamic-pituitary involvement with a central disturbance in the frequency and amplitude of gonadotropin releasing hormone (GnRH). As such, factors that affect GnRH secretion must also be considered in the pathogenesis of PCO, including endogenous opioids, neurologic factors, and prolactin.[7]

A strong association between galactorrhea, hyperprolactinemia, and PCO has long been recognized. Many reports have suggested that hyperprolactinemia in PCO is caused by the inhibitory effect of increased estrogen levels on the dopaminergic system.[7] Support of this concept is demonstrated, in part, by a sustained reduction in circulating LH levels achieved in PCO patients administered dopamine.[8] Further studies have shown a synchrony between endogenous pulses of LH and prolactin,[7] with bromocriptine (a dopamine agonist) induced reductions of prolactin and LH secretion in PCO patients.[9] Moreover, hyperprolactinemic women demonstrate significantly higher levels of dihydroepiandrosterone-sulfate (DHEAS) than normal controls, with treatment of the elevated prolactin resulting in DHEAS reductions.[7]

Bilateral polycystic ovaries represent the sine qua non of PCO. Typically, follicles in the ovaries are arrested in follicular development,

measure approximately two to eight mm in diameter and appear as a "string of pearls" located beneath a smooth, glistening capsule. In contrast with normo-ovulatory patients, follicles in PCO patients are usually found to be in varying stages of growth and atresia, without production of a dominant follicle.[7] Erickson et al., demonstrated that arrested follicular development in PCO was not due to an inherent defect in ovarian steroidogenesis. In this study, the follicles of PCO patients were capable of producing normal levels of estrogen from the precursor androstenedione in the presence of appropriate levels of gonadotropin secretion or the addition of exogenous FSH.[10]

Polycystic ovary syndrome often presents with excessive hirsutism and hyperandrogenism. The adrenal gland, as a producer of approximately 50% of peripheral androstenedione (the remaining 50% being of ovarian origin), may be significantly involved in the pathogenesis of a proportion of PCO patients. Congenital or acquired blocks in the androgen steroidogenesis pathway may lead to elevations in levels of precursor hormones with androgenizing potential, resulting in hirsutism, acne, oligomenorrhea, infertility, and other findings similar to those in PCO. However, most investigators agree that this PCO-like appearance in congenital adrenal hyperplasia is distinct from "classical" PCO. The adrenal gland is normally regulated by a tight feedback loop involving the hypothalamus and pituitary. As such, the association between adrenocorticotropic hormone (ACTH) and PCO has become a subject of much interest.[7] Although PCO patients usually have higher levels of LH, dihydroepiandrosterone (DHEA), DHEAS, testosterone, and androstenedione than healthy women, ACTH levels are similar in both groups.[11] This finding introduces the possibility that adrenal androgen production in PCO patients may be due to altered adrenal responsiveness to ACTH, rather than to alterations in ACTH production.[7]

Of great importance in the pathogenesis of PCO in many patients are the peripheral extraglandular sources of circulating estrogens. Although the majority of estrogens originate from the ovaries and adrenal glands, aromatization of androgens to weaker estrogens occurs in the skin, brain, and adipose tissue. Because PCO patients are often overweight, the increased peripheral aromatization of elevated androgens may lead to excessive levels of estrogens. The increased estradiol levels have, in turn, been shown to correlate with both high LH/FSH ratios[7] and reductions in hypothalamic dopamine levels in women with PCO.[12]

The most common medication used in the ovulation induction of patients with PCO is clomiphene citrate (CC; Clomid), a partial agonist of estrogen. Using CC, approximately 80 to 90% of PCO patients achieve ovulation, with 40 to 50% of these women ultimately conceiving. In patients with poor ovulatory response to CC, the treatment of choice is usually human menopausal gonadotropin (hMG).[7]

Ovulation induction in patients with PCO is associated with an increased risk of multiple births and ovarian hyperstimulation syndrome (characterized by massive ovarian enlargement and third-space fluid accumulation), possibly due to the presence of multiple small follicles and elevated LH and estrogen levels. Therefore, in PCO patients, pregnancy rates are maximized and complication rates minimized by administering gonadotropins in small doses over a longer period of time than in the ovulation induction of other infertility patients.[7]

4. Male Factor Infertility

In approximately 30 to 40% percent of infertile couples, male factors are at least partially responsible for the inability to conceive.[13] In one third of

infertile couples, male-factor abnormalities are detected in the man alone, while in an additional 20% of cases, etiologic factors are diagnosed in both the man and woman.[14, 15] As part of a standard evaluation, a semen analysis should be undertaken in all couples presenting with infertility. Although there is wide variation between patients and repeat samples from the same patients in terms of volume, number, and motility of sperm, normal guidelines for each of these parameters are well established.[2] Typical abnormalities detected by semen analysis are low sperm concentration (oligospermia), decreased motility (asthenospermia), decreased normal forms (teratospermia), and increased semen viscosity.[16] If an abnormality is found, a repeat analysis should be performed two to three months later in order to determine the actual presence or absence of a male factor. It is considered clinically inappropriate to designate a male patient as infertile based upon a single semen analysis.[2] Examination of the male with abnormal semen analyses may reveal a varicocele or ductal obstruction (both of which are surgically correctable), an immunologic disorder, a hormonal disturbance, or after thorough negative evaluation, an idiopathic etiology. When treatment of the male partner is unsuccessful, insemination with split ejaculates, intrauterine insemination with washed sperm, or combinations of these modalities with ovulation induction may be effective. In cases of severe sperm abnormalities, conventional IVF is often useful, given its ability to combine high concentrations of motile sperm with oocytes in a small volume of medium, optimizing the possibility of fertilization. Fertilization rates are uniformly reduced in male factor infertility. Although embryo transfer rates are approximately 50%, pregnancy rates per transfer are similar to those for other diagnostic etiologies of infertility.[16]

5. Unexplained Infertility

The term "unexplained infertility" is reserved for couples experiencing infertility of greater than two years' duration in the face of a normal, complete evaluation, including documentation of normal ovulation, normal semen parameters, normal sperm-mucus interaction and post-coital test, and normal uterus and tubes by HSG and laparoscopy.[17] A study conducted by Barnea et al. demonstrated that among patients with unexplained infertility diagnosed by such an evaluation, the average duration of infertility was 39 months, with 34% achieving pregnancy within six months of study registration and 80% within five years.[18]

Many explanations for the etiology of unexplained infertility have been postulated, including changes in the "ovarian reserve" and other occult abnormalities. An increase in the proportion of couples assigned to the unexplained category of infertility may, in part, be a consequence of changing childbearing behavior, with more women delaying childbearing to older ages.[17] With older chronological female age may come alterations in the ovarian response, as indicated by a study performed on 51 women aged 35 or older with unexplained infertility. In this study, a clomiphene challenge test was used to assess fertility, with women subsequently divided into two groups: those with diminished ovarian reserve (with post-challenge FSH levels elevated to a mean of 39 mIU/ml) and those with adequate reserve (with post-challenge mean FSH levels of 12 mIU/ml). Treatment consisting of ovulation induction, timed intercourse, or insemination resulted in pregnancy in 42% of women with adequate reserve, while only 5% of those with diminished reserve became pregnant.[19] Other explanations for unexplained infertility may include occult dysfunction of ovum production and release, defective corpus luteum function, defects in sperm transport and

function (with otherwise normal semen parameters), subtle uterine or tubal defects including dysfunctional ovum pick-up and transport, "silent" infection in either the seminal fluid or the female genital tract (chlamydia, mycoplasma, and ureaplasma being commonly associated with subfertility), and immunologic infertility.[17]

Treatment for true unexplained infertility, as confirmed by a more detailed evaluation (ultrasound evaluation of follicular dynamics, sperm function assays, evaluation of subtle tubal, uterine, and pelvic factors, tests for occult infectious agents, and immunologic testing) usually includes ovulation induction and assisted reproductive technology in the form of gamete intrafallopian transfer (GIFT) or IVF .[17]

C. Ovulation Induction in IVF

The early use of superovulation cycles in IVF established that success was principally determined by the number of embryos transferred into the uterine cavity. In essence, the replacement of three or more healthy embryos optimized the likelihood of achieving an ongoing pregnancy. Replacement of more than three embryos increases the likelihood of multiple pregnancies while not increasing the pregnancy rate.[20] Given this information, it has become usual practice to recover atleast four or five good quality oocytes in order to be reasonably sure of the replacement of three healthy embryos.[21] Although natural cycle IVF may be of value in a limited number of patients, it has been predominantly superseded by superovulation cycles for assisted reproduction in an attempt to maximize the generation of large numbers of mature oocytes. What follows is a review of normal folliculogenesis in the unstimulated cycle and an overview of the ovulation induction procedures commonly used in IVF.

1. Folliculogenesis

Ovarian function in the normal or stimulated cycle rests upon communication, of either a stimulatory or inhibitory nature, between the hypothalamus, pituitary, and ovary. During supraphysiologic stimulation of the ovaries for *in vitro* fertilization, the pharmacology of exogenous hormone therapy markedly alters the relationships between these endocrine organs, affecting the dynamics of follicular development.[22]

During the embryonic period, primordial germ cell populations are established that will later give rise to oocytes. Just prior to birth, the two to four million primary oocytes formed during fetal life reach prophase of the first meiotic division. The primordial follicle then consists of this primary oocyte covered by a single layer of granulosa cells.[23] A portion of the primordial follicles begin to grow or undergo atresia as soon as they are formed, while others enter a quiescent state until puberty.[24] Reactivation of quiescent primordial follicles, follicular development, and oocyte atresia appear to punctuate a continuous process in the ovary, beginning immediately after the first follicles form and progressing until the end of the reproductive period.[25]

Hodgen proposes a two-tiered ovarian mechanism operative in follicular development. At the first tier, specific factors, both intraovarian and extraovarian, govern the progressive diminution of the cohort of developing follicles to the ovulatory quota of each cycle. This regulation of cohort size only operates, however, when circulating gonadotropins (FSH and LH) exceed minimal tonic levels. At the second tier, ovarian steroidal and nonsteroidal hormones negatively feed back to inhibit gonadotropin secretion, thus controlling circulating gonadotropins at appropriate tonic levels. The first and second tiers of the proposed mechanism are intimately

connected, such that changes in the components of one tier may drastically alter activities of the second. For example, it is hypothesized that if circulating gonadotropin levels are far above the tonic set-point, then first tier ovarian mechanisms become impaired, resulting in superovulation. Therefore, multiple follicular development occurs as a result of both increased gonadotropin availability and the overriding of early follicle selection mechanisms.[22]

During each cycle, primordial follicles are "recruited" and depart from the resting pool to undergo a well-characterized pattern of development,[22] termed by Schwartz the "trajectory of follicle growth".[26] Growing follicles are vulnerable to atresia at any point along this "trajectory", making recruitment an obligatory, but decidedly preliminary, step in the selection of a dominant follicle from the developing cohort.[22]

Under the influence of FSH in the natural cycle, the number of granulosa cells in the primordial follicle increases and the follicle matures into a primary or preantral follicle. As the number of granulosa cells increases, the production and secretion of estradiol from these cells increases, representing the first sign of functional maturation.[23, 24] Estradiol, in turn, stimulates preantral follicle growth, prevents follicular atresia, and potentiates the effects of FSH on the granulosa cells. The follicle destined to become dominant secretes the greatest amount of estradiol; in fact, almost 80% of the approximately 500 mg of estradiol produced daily just prior to ovulation originates from the dominant follicle. The elevated estradiol levels increase the density of FSH receptors on the follicle surface, ultimately causing a surge in the mitotic activity of the granulosa cells. Moreover, the rising concentration of estradiol exerts a negative feedback effect on the release of FSH from the pituitary, halting the development of the less FSH

sensitive non-dominant follicles. The dominant follicle, in contrast, continues to develop due not only to its increased highly vascularized theca cells, allowing more FSH to reach the receptors, but also to its increased density of FSH receptors. As the granulosa cells proliferate, LH receptors appear on their surface membranes in response to FSH and estradiol; when LH binds to the receptors, granulosa cell proliferation ceases and the cells begin to secrete progesterone.[23] In essence, the follicle destined to ovulate plays a pivotal role in regulating the size of the ovulatory quota, inhibiting the development of other follicles in the cohort, and becoming "dominant" about a week prior to ovulation. Interestingly enough, this dominant follicle continues to thrive in a physiologic milieu it has itself made inhospitable for others.[22]

Just prior to ovulation, the dominant follicle attains, on average, a mean diameter of approximately 19.5 mm, with a range of 18 to 25 mm. The maximal size of the dominant follicle can vary among different women, as well as in the same woman in different cycles. Rapidly rising estradiol levels, in combination with the small increase in progesterone produced by the dominant follicle, serve as the signal to the hypothalamic-pituitary axis that the follicle has matured sufficiently for ovulation. At midcycle, the substantial estradiol increase stimulates LH secretion and the small preovulatory progesterone increase stimulates FSH secretion, culminating in an LH and FSH surge. The midcycle LH surge is responsible for initiating the ovulatory process, germinal vesicle disruption, and completion of metaphase I of meiosis. LH stimulates synthesis of prostaglandins and proteolytic enzymes, both of which aid in follicular rupture; FSH stimulates production of plasminogen activator, producing plasmin to detach the cumulus from the granulosa cells, assisting egg extrusion during follicular rupture. It has been

determined that ovulation occurs approximately 24 hours after the estradiol peak, 12 to 16 hours after the LH peak in serum, and about 32 hours after the initial elevation in LH level. Once the oocyte is extruded during follicle rupture, the corpus luteum forms and produces progesterone under the influence of LH. After ovulation, the increasing levels of estradiol and progesterone exert a negative feedback effect on FSH and LH, respectively.[23]

Although the precise mechanisms of folliculogenesis during ovulation induction have still not been fully elucidated, it is generally postulated that follicular recruitment occurs in much the same fashion as it does during the natural cycle. The most notable difference between stimulated and non-stimulated cycles remains, quite simply, the sheer number of follicles recruited for ovulation, with higher yields for stimulated cycles.

2. Clomiphene Citrate

Until recently, the most frequent ovarian stimulation regimen used in assisted conception was the combination of clomiphene citrate and exogenous gonadotropins (hMG). The purpose of such a regimen is to override the estrogen-driven negative feedback on endogenous gonadotropin secretion that occurs in a natural cycle. In essence, CC causes a mild hypersecretion of pituitary gonadotropins, thus stimulating the recruitment of a number of small follicles. Exogenous gonadotropins may be administered thereafter to sustain the growth of this cohort of recruited follicles, achieving multiple synchronous follicular development. Multiple follicular development causes increased estradiol levels, resulting in positive feedback elevations in LH. The best success rates with the CC/hMG combination have been achieved with monitoring for the endogenous LH surge, which may occur in the late follicular phase. If the endogenous LH surge remains undetected, ovulation

prior to oocyte recovery or the collection of postmature oocytes may occur. The occurrence of the LH surge is not predictable by follicle size once the leading follicle exceeds 15 mm in diameter, by the absolute level of estrogen, or by the rate of estrogen rise. If the LH surge is successfully detected, used to time oocyte recovery, and supported by the administration of human chorionic gonadotropin (hCG), the patient's clinical performance through an assisted conception cycle is optimized. The criteria for the administration of hCG for ovulation induction in CC/hMG cycles are the detection of the spontaneous LH surge or one or more follicles reaching 18-20 mm in diameter.[27] If hCG is given too late, the most advanced follicles may yield postmature (or fragmented) eggs of low potential viability; conversely, if hCG is injected too early, the eggs may be immature.[22] Oocyte recovery is timed approximately 32 hours after 5,000-10,000 IU of hCG is administered.[27]

Early results with CC/hMG demonstrated a clinical pregnancy rate of 35% following the transfer of three embryos, clinical pregnancy rates per transfer of 25%, and a "take-home baby rate" of approximately 15%. A comparison of patients who became pregnant after embryo transfer and those whose embryos failed to implant revealed that patients who established a clinical pregnancy had both significantly lower urinary LH output in the 2 days prior to ovulation induction and lower plasma LH levels in the late follicular phase. More specifically, higher late follicular phase LH levels correlated with compromised oocyte quality and fitness, reduced implantation rate, and early pregnancy loss. The degree of gonadotropin hypersecretion induced with clomiphene appears to vary between individuals and between cycles within the same individual.[27]

The consequences of high late follicular phase LH levels may be explained by consideration of the mechanism of oocyte maturation and

ovarian paracrinology. One hypothesis states that the natural midcycle LH surge promotes the degradation of the gap-junctional communication system in the granulosa cell layers, thus impeding the passage of oocyte maturation inhibitor(s) from the follicle wall to the oocyte.[28] A second hypothesis postulates that the LH surge stimulates the expression of maturation-inducing factors by the granulosa cells.[29] It has been shown that high basal LH concentrations, as are present in individuals with PCO, may have direct negative influences upon oocyte viability in ovarian stimulation for IVF[30,31], although other evidence has failed to generate support of this.[32]

3. Gonadotropin Releasing Hormone Agonists

In patients who show inappropriately elevated levels of LH during superovulation with CC/hMG, improvements in follicular recruitment, fertilization rates, and pregnancy rates can be achieved through an inhibition of LH by administration of gonadotropin releasing hormone (GnRH) agonists. Upon first exposure to the GnRH agonist, a massive release of the pituitary gonadotropins LH and FSH occurs for approximately two to four days, followed by the requisite feedback inhibition of LH synthesis and depletion of its readily releasable form. The membrane-bound GnRH agonist stimulates receptor internalization and degradation, thus decreasing receptor numbers; moreover, the continued occupation of the pituitary gonadotropin receptors maintains a lowered sensitivity to endogenous GnRH. The consequence of prolonged GnRH agonist use is lowered circulating levels of LH (and FSH, to a smaller degree) as well as inhibition of ovulation. After cessation of GnRH analogue administration, normal pituitary function is only recovered as *de novo* receptor synthesis occurs. As the gonadotropin

receptors become sensitive to endogenous GnRH, circulating levels of LH and FSH are restored.[27]

Ovulation induction strategies combining GnRH analogues and exogenous gonadotropins (hMG or pure FSH) fall into three basic categories: long, short, and ultra-short protocols. In the long protocol, a GnRH agonist is administered for a period of 8 to 21 days, beginning in the early to mid-luteal phase of the preceding cycle, achieving a temporary state of hypogonadotropic hypogonadism. Once desensitization of the pituitary gonadotrophs to endogenous GnRH is completed, the GnRH agonist dose is reduced to a maintenance level, prolonging ovarian quiescence and suppression of circulating gonadotropins while preventing an endogenous LH surge. Adequate superovulation may then be achieved by the daily administration of exogenous gonadotropins, with approximately 10 days of stimulation being adequate for sufficient ovulation induction and estrogen priming of the endometrium. Due to the suppression of endogenous LH secretion, luteinization rarely occurs until an ovulation-inducing dose of hCG is given.[27]

The short and ultra-short regimes both make use of the initial release or "flare" phase of GnRH agonist action, stimulating follicular recruitment and early development[27] and reducing the amount of gonadotropin required.[33] As the pituitary gonadotrophs become desensitized by prolonged GnRH agonist administration and hyposecretion of endogenous gonadotropins occurs, continued follicular growth is supported by exogenous gonadotropins. In order to take advantage of the release phase, both short protocols must commence in the early follicular phase, by day two or three of menstruation. It is important to note, however, that the onset of menses does not uniformly correlate with the complete demise of the corpus luteum

from the previous cycle.[27] As such, the corpus luteum may be "rescued" by the endogenous gonadotropin release following agonist administration, causing elevated levels of progesterone through the follicular phase at a time when the endometrium should be proliferating without exposure to progestational influences.[33]

The use of GnRH agonists, in combination with exogenous gonadotropins, significantly increases the chance of pregnancy when compared to treatment with clomiphene citrate combined with hMG. LH suppression in the late follicular phase results in improved oocyte and embryo quality.[27]

D. Factors Affecting the Outcome of IVF

1. Age

The outcome of *in vitro* fertilization and embryo transfer (IVF-ET) is influenced by multiple factors. According to a prospective study undertaken by Hughes et al., the single factor most significantly detrimental to pregnancy rates in IVF was advancing female age, with a linear decline beginning at 25 years.[34] Studies evaluating the effect of increasing female age on IVF outcome have yielded varying estimates of pregnancy rates, with women aged 40 years or more achieving conception in anywhere from 3.8%[34] to 23%[35] of cycles. Moreover, patients in the higher age group demonstrate a decreased ability to maintain pregnancies, with spontaneous abortion rates observed to be as high as 60%.[35] It is generally accepted that aging female infertility patients have considerably worse IVF outcomes than younger diagnosis-matched patients.

2. Male Factor Infertility

Studies performed on patients with male factor infertility have shown a distinct relationship between sperm factors and IVF-ET outcome. Sperm motility and morphology, concentration of sperm in the insemination medium, and sperm vitality have all correlated with fertilization rate.[36, 37]

3. Embryo Quality

Although definitions of embryo quality and embryo grading systems differ among IVF centers, both morphologic appearance and cleavage rate have been clearly correlated with pregnancy rate. Puissant et al. defined a semi-quantitative, non-invasive method for scoring embryos based upon the quantity of anucleate fragments expelled by the embryos at cleavage and the embryo development rate. Healthy appearing embryos were more often associated with pregnancy, and in particular, with multiple pregnancy.[38]

4. Patterns of Estradiol Secretion

Pregnancies resulting from IVF-ET cycles have been associated with wide ranges of estradiol values. There is considerable controversy regarding appropriate levels of estradiol during the various phases of IVF. As shown by Jones et al., women classified as high, normal, or low responders with respect to estradiol levels showed only small differences in pregnancy rates. It was therefore concluded that the absolute estradiol level was generally a poor predictor of success in IVF.[39] However, in a study conducted by Dor et al., changes in the daily pattern of estradiol during the peri-hCG period were found to be a reliable predictor of outcome in cycles of women stimulated with CC/hMG or hMG alone. Although there were no differences in the mean daily follicular phase estradiol levels between the pregnant and control groups, failure of fertilization was associated with significantly lower estradiol

levels during the days just prior to and following the administration of hCG. In fact, 96% of the women in the study who achieved conception demonstrated either rising or plateauing estradiol levels on the day following hCG (seen in only 62.5% of control cycles), while 37.5% of the women in the control, non-pregnant group had decreasing estradiol levels during the same time period. If declining estradiol is interpreted as indicative of follicular atresia, then it is plausible that lower estradiol levels in the post-hCG period could result in a poorer quality oocyte and a correspondingly diminished likelihood of fertilization and conception.[40] In contrast to the Dor et al. study, Howles et al. showed that plasma levels of estradiol four and six days after oocyte recovery during CC/hMG stimulated cycles were significantly higher in patients who did not become pregnant than in the pregnant group,[41] supporting the possibility that elevated estradiol levels after hCG administration may be detrimental to establishing a pregnancy.

5. Endometrial Receptivity

The establishment of pregnancy through the use of IVF depends not only on the generation of multiple embryos of good quality, but also upon the presence of a receptive endometrium to allow and maintain embryonic implantation. While stimulating adequate numbers of oocytes for fertilization, ovulation induction simultaneously causes the production of supraphysiologic levels of estradiol and progesterone from the multiple follicles and subsequent corpora lutea. The extraordinarily high steroid levels encountered in the early luteal phase during ovarian stimulation must undoubtedly influence the quality and receptivity of the endometrium. In fact, some studies suggest that the balance or ratio of steroid hormones, and their subsequent influence on the endometrium, may be aberrant in women

who fail to implant.[41] Along these same lines, conflicting discussion abounds in the literature regarding the necessity for or detriment of high progesterone output during the luteal phase of stimulated cycles. Supplementation of the luteal phase with exogenous progesterone and stimulation of endogenous progesterone production by the corpus luteum with hCG are popular practices in IVF, indicating the belief that higher luteal phase progesterone may positively impact endometrial quality and implantation success.[27] Studies performed to date, however, have not consistently demonstrated that luteal phase support increases subsequent pregnancy rates.

6. Oocyte Quality

The quality of the oocyte obtained in an IVF program has been shown to be one of the keys to determining oocyte maturity and embryo quality, both of which influence normal fertilization and subsequent development. Premature or postmature insemination of oocytes have been demonstrated to result in a higher incidence of failed or abnormal fertilization.[42] Despite this, the optimal diameter of the preovulatory follicle has not been precisely determined. Most likely, the optimal oocyte measures between 18 and 20 mm in diameter before ovulation and 26 to 28 mm soon after ovulation begins, around the time of oocyte retrieval.[43] At the time of follicular puncture, the retrieval of smaller follicles frequently occurs. It has been shown, however, that oocytes derived from large follicles are more efficient with respect to resumption of meiosis and fertilizing ability. In a study by Bomsel-Helmreich et al., no nuclear maturation was found in ova of follicles measuring less than 16 mm, highlighting the delay in maturation among smaller follicles.[44]

7. Number of Oocytes Retrieved

Many studies have examined the relationship between the number of oocytes retrieved during a cycle of IVF and subsequent fertilization and outcome of embryo transfer. Pellicer et al. demonstrated that the fertilization rate was significantly decreased in groups of patients producing 11 or more oocytes in comparison with patients producing smaller numbers of oocytes at the time of collection.[45] Testart et al. have also found a lower fertilization and pregnancy rate when the number of follicles increases, with the proportion of normal embryos per recovered oocyte inversely related to the degree of ovarian response.[46] In another study by Sharma et al., the clinical pregnancy rate per embryo transfer showed a linear rise when up to 5 oocytes were collected, with the rate plateauing at higher oocyte numbers. Moreover, this study demonstrated that the success of implantation (defined as the number of gestational sacs ÷ number of embryos transferred x 100) was highest when 7 to 9 oocytes were retrieved and more than 60% of these fertilized; when 10 or more oocytes were collected, the implantation rate revealed a progressive decline, even though the fertilization rate remained above 60%.[47]

8. Number of Preovulatory Follicles

The relationship between the number of follicles and the occurrence and quality of pregnancy has also received attention. Testart et al. demonstrated the influence of ovarian response on oocyte suitability for transfer in cycles with the transfer of one, two, or three embryos. In this study, the number of punctured follicles, recovered oocytes, and cleaved embryos had no significant effect on the success of embryo transfer in patients who received one, two, or three embryos, although the proportion of ongoing

pregnancies decreased when the number of follicles increased. Moreover, a higher oocyte cleavage rate was demonstrated among patients with one to four follicles (termed a "moderate" response) than among those producing five or more follicles.[48] In a study conducted by Forman et al., the pregnancy rate in a population combining all infertility diagnoses was shown to decrease from 52% with ≤ 2 follicles measuring more than 14 mm in diameter on the day of hCG administration to 33% with 3 to 4 follicles and 20% with 9 or more follicles detected ($P<0.01$). Surprisingly, the authors of this study concluded that the number of follicles measuring greater than 14 mm at the time of hCG did not correlate with pregnancy rate, although the data suggest otherwise.[49]

Of the studies cited here, it is apparent that a relationship exists between the number of preovulatory follicles and the outcome of ET, observed both as a trend[48] and as a statistically significant result. [49] However, it is essential to note that these correlations were demonstrated in populations combining patients with many etiologies of infertility. Whether the relationship between ovulatory response and pregnancy rate is borne out in groups of patients with specific diagnoses has not yet been determined.

III. STATEMENT OF PURPOSE

The purpose of the study proposed here is an examination of the relationships between pretransfer parameters in IVF-ET and the subsequent occurrence of pregnancy in patients assigned to individual diagnostic groups, with particular focus on patients with tubal disease.

IV. METHODS

A. Overview of the Population

The study evaluated 505 randomly selected cycles of IVF performed between February 1984 and November 1993. Cases used in the study included those with successful ovarian response, fertilization, and embryo transfer, as well as those in which no fertilization or transfer of retrieved oocytes occurred. The only cycles omitted during chart review were those dropped before adequate ovarian stimulation could be completed due to poor response (few follicles, low estradiol), or an exaggerated response to stimulation (excessive follicular development, high estradiol, risk of ovarian hyperstimulation syndrome).

B. Ovulation Induction Protocols

Controlled ovarian hyperstimulation was primarily achieved with human menopausal gonadotropin (hMG; Pergonal) formulated as ampoules containing 75 IU each of FSH and LH, or with pure FSH containing 75 IU of FSH alone (pure FSH/Metrodin). In the majority of cycles included in the study, hMG or pure FSH was used either alone or in combination with each other and/or with a GnRH agonist (Lupron). The remainder of cases were stimulated using Clomid protocols, either alone or in combination with hMG. The daily doses of hMG/FSH administered to each patient per cycle varied and were adjusted according to diagnosis, folliculogenic response, and past cycle experience.

Monitoring was performed both ultrasonographically and biochemically. Baseline ultrasound scans were performed before hMG/FSH administration to detect ovarian cysts and fibroids, as well as to ensure

adequate ovarian suppression. Monitoring beyond this baseline scan was then individualized, with subsequent endovaginal ultrasound exams and estradiol levels dictating changes in management.

On the day of hCG administration, each patient had an estradiol and progesterone level drawn and an endovaginal ultrasound scan performed. The standard criteria for the administration of hCG (10,000 IU) included ultrasonographic detection of at least 2 follicles measuring greater than 16 mm in diameter, and an estradiol level not exceeding 3,000 pg/ml. Oocyte retrievals were carried out approximately 32 hours after hCG administration.

No oocytes were excluded from the insemination protocol. All oocytes were examined 16 to 18 hours after insemination and again at 48 hours, just prior to embryo transfer; if, at these times, oocyte cleavage or two pronuclei were visualized, fertilization was considered to have occurred. Upon examination, embryos were scored to indicate quality, maturity, and suitability for transfer. The embryo scoring system used in cycles included in this study was defined as follows: grade 1 was assigned to unfragmented embryos with regular blastomeres; grade 2 was assigned to embryos with minor fragments and regular blastomeres; remaining embryos with evidence of irregular blastomeres or more severe fragmentation were assigned a grade of 3. Embryos were transferred 2 days after oocyte retrieval. Variable numbers of embryos were transferred and frozen in each cycle, based upon the number and quality of oocytes and embryos produced, as well as upon the patient's age, past reproductive history, and plans for fetal reduction in the case of multiple gestation.

Plasma radioimmunoassays to detect embryonic hCG secretion were performed on the twelfth day after embryo transfer. Clinical pregnancy was documented by detection of fetal heart activity on ultrasound scan.

C. Etiologic Definitions

Patients were considered to have tubal factor infertility if the fallopian tubes were shown to be blocked, either unilaterally or bilaterally, by HSG or laparoscopy. All cases of severe tubal scarring or encasement in adhesions evident upon laparoscopy were accompanied by abnormal hysterosalpingograms, and as such, were easily interpretable as tubal factor infertility. Patients with recurrent ectopic pregnancies and pelvic adhesions diagnosed by laparoscopy, with or without HSG documentation of definitive blockage, were also considered to have tubal disease. Patients were determined to have "pure" tubal disease in the presence of a normal semen analysis, regular ovulations documented by a history of regular cycles, positive LH predictor kits, or biphasic basal body temperature (BBT) charts, and the absence of endometriosis and PCO.

The diagnosis of polycystic ovary syndrome was made based upon a combination of a number of factors, including a history of oligo- or anovulation, an ultrasound scan revealing enlarged ovaries with multiple small follicles, hirsutism, and hyperandrogenism indicated by increased DHEA, DHEAS, and free testosterone levels. Based on the rationale that the presence of PCO dominated the clinical picture of patients with this disorder, patients were assigned to the PCO diagnostic group with or without the presence of other etiologies of infertility.

Patients diagnosed with endometriosis were designated as such based upon laparoscopic documentation of the presence or absence of ectopic endometrial implants, categorized according to the American Fertility Society classification.[50] Stage I of the classification was designated as minimal endometriosis, stage II as mild, stage III as moderate, and stage IV as severe, for the purposes of this study. Patients grouped in the "pure" endometriosis

category were required to have endometriosis documented by the previously described classification, and an absence of tubal disease, male factor, and PCO; "pure" endometriosis patients were, however, allowed to have other diagnoses not expressly evaluated in this study (luteal phase defects or hypothalamic anovulation).

The diagnosis of male factor was applied to couples based upon stringent seminal criteria. In order to be judged subfertile, the semen analysis was required to demonstrate a sperm count less than 20 million/ml, sperm motility less than 40%, or a semen volume less than 1.5 ml. In addition, abnormal creatinine kinase parameters were considered convincing indications of male subfertility,[51,52] even when accompanied by otherwise normal semen parameters. "Pure" male factor patients were designated as such if clinical records indicated a presence of male factor with an absence of tubal disease, PCO, or endometriosis.

Patients were assigned to the unexplained infertility group in the presence of persistent infertility without evidence of ovulatory dysfunction, tubal disease, endometriosis, or male factor. In addition, all unexplained infertility patients had confirmed normal luteal function, determined by an endometrial biopsy taken on day 26 or 27 of the natural cycle (the glands and stroma of the tissue biopsied must be histologically in-phase with the cycle day upon which it was sampled, as determined by the onset of next menses).

D. Statistical Methods

Unless stated otherwise, all variables were described as means $\pm$ standard error of the mean. Differences between means were tested by Student's *t*-test. Correlations between variables in each cycle were analyzed using Pearson's correlations.

To assess the significance of heterogeneities in frequency distributions among groups and subgroups, chi-square tests were used. If the expected frequency in any subgroup was less than five, the Fisher exact test (two-tailed) was used instead.

E. Contributions

The original study design and concept was provided by Dr. David L. Olive and Dr. Antoni J. Duleba. Data collection was performed by Nicole Hausman. Statistical analysis was performed by Nicole Hausman under the supervision of Dr. Duleba.

V. RESULTS

A. Population Distribution

The initial population examined in this study consisted of 208 women, from which data on 505 IVF cycles were obtained. The study focused on the analysis of patients who underwent controlled ovarian hyperstimulation. In order to examine such patients, stimulated cycles (N=466 cycles) were separated from natural (N=18 cycles), egg donor (N=5 cycles), and sperm donor (N=16 cycles) cycles; the demographics and distribution of each of these subdivisions are shown in Table 1 and Figure 1, respectively. As shown in Table 2 and Figure 2, the general (stimulated) population was further subdivided by etiology of infertility into: tubal disease (N=106 cycles), PCO (N=34 cycles), endometriosis (N=36 cycles), male factor (N=28 cycles), and unexplained infertility (N=36 cycles), comprising 23%, 7%, 9%, 6%, and 8% of the general population, respectively. The remaining 47% (N=226 cycles) of the general population consisted of patients with more than one of the above primary diagnoses or other diagnoses (luteal phase defects, hypothalamic anovulation). The mean ages of patients in the individual diagnostic categories were not significantly different.

The distribution and demographics of the various ovarian stimulation protocols used in the general population are summarized in Table 3. Among those who underwent ovarian stimulation, 22.5% of patients received hMG/FSH only, 71.3% received hMG/FSH and Lupron, and 6.3% received Clomid, with or without hMG.

B. Outcome of IVF

The means of individual variables assessed during the IVF cycles in each diagnostic category are presented in Table 4. The average duration of

gonadotropin administration in individual diagnostic categories ranged from 8.19 to 9.58 days of hMG/FSH stimulation, and from 21.88 to 31.65 ampoules of hMG/FSH used. Of note, the cycles comprising the PCO category demonstrated the longest period of hMG/FSH stimulation (9.58 $\pm$ 0.66 days) with the lowest amount of hMG/FSH used (21.88 $\pm$ 1.21 ampoules), corresponding to the widely accepted practice of slow, low dose stimulation of PCO patients.

The average number of oocytes retrieved in each diagnostic category also varied widely, ranging from 7.79 in the male factor group to 12.18 in the PCO group. The average number of retrieved oocytes in PCO patients was 49% greater than the number of oocytes retrieved in the general population; however, this increase was associated with only a 20% increase in the number of fertilized oocytes in the PCO group above the general population. Furthermore, PCO patients had the lowest probability of fertilization, averaging 47.59%. Patients in the tubal disease population had the highest percentage of oocyte fertilization, averaging 74.44%.

The average number of transferred embryos ranged from 2.65 in those with male factor to 4.08 in those with endometriosis. In addition, the average grade of transferred embryos ranged from 1.85 in patients with PCO to 2.14 in patients with endometriosis, noting that lower grades were associated with higher quality, as described in the Methods section. Thus, patients with PCO and tubal disease had the highest quality of transferred embryos, with grades of 1.85 and 1.86, respectively.

Clinical pregnancy rate was 17.6% for the general population, 19.8% in the tubal disease category, 20.6% in patients with PCO, 13.9% in those with endometriosis, 10.7% in the male factor group, and 19.4% in patients with unexplained infertility (Figure 3).

The mean number of follicles measuring ≥ 12 mm in diameter on the day of hCG administration was 7.51 in the general population, 7.34 in the tubal disease category, 9.39 in patients with PCO, 8.78 in those with endometriosis, 8.16 in the male factor group, and 7.31 in patients with unexplained infertility (Figure 4).

The average estradiol levels measured on the day of hCG administration ranged from 1,275 pg/ml in the male factor group to 2,122 pg/ml in the PCO group (Figure 5).

C. Correlations

The correlations between the number of follicles measuring ≥ 12 mm in diameter on the day of hCG administration and several of the variables analyzed in each cycle of IVF are shown in Table 5. In general, positive correlations were observed between the number of follicles and estradiol levels, number of oocytes retrieved, number of oocytes fertilized, and number of embryos transferred. A significant negative correlation was observed between the number of follicles ≥ 12 mm and the percentage of oocytes fertilized in the general and tubal disease groups, with the stronger correlation seen in the tubal disease group. While a slight negative correlation between these parameters was noted in the endometriosis and unexplained infertility categories, these effects were not statistically significant. Furthermore, there was a trend in the tubal disease group toward an increase in the grade of transferred embryos (i.e. decrease in embryo quality) with increasing number of follicles measuring ≥ 12 mm. This correlation was not seen in any other diagnostic category.

The correlations between the number of oocytes retrieved and several of the parameters studied in each cycle of IVF are shown in Table 6. The

duration of administration of hMG/FSH and total amount of hMG/FSH used per cycle appeared to correlate negatively with the number of oocytes retrieved in almost all diagnostic groups. In all diagnostic groups, positive correlations were observed between the number of oocytes retrieved and estradiol level, number of oocytes fertilized, and number of embryos transferred. Statistically significant negative correlations between the number of oocytes retrieved and the percentage of oocytes fertilized were observed in both the tubal disease and endometriosis populations. Furthermore, the embryo transfer grade increased with increasing number of oocytes retrieved for both tubal disease and PCO patients, although neither relationship was statistically significant.

D. Influence of the Number of Follicles on Pregnancy Rate

Figure 6 presents pregnancy rate in the individual diagnostic categories as a function of the number of follicles measuring ≥ 12 mm in diameter on the day of hCG administration. The patients in each diagnostic category were stratified into two groups: those producing ≤ 7 follicles measuring ≥ 12 mm and those producing > 7 follicles measuring ≥ 12 mm. In the general population, there was virtually no difference in pregnancy rate as a function of number of follicles detected on the day of hCG, with an 18.4% pregnancy rate (46 of 250 cases) for ≤ 7 follicles and a 16.5% pregnancy rate (35 of 212 cases) for > 7 follicles. However, in the tubal disease group, a statistically significant decline in pregnancy rate was demonstrated between the two stratifications, with pregnancy rates of 28.3% (17 of 60 cases) for patients with ≤ 7 follicles and 8.7% (4 of 46 cases) for patients with > 7 follicles (Fisher exact test, $P = 0.014$). In contrast, the PCO and unexplained infertility groups

displayed the opposite trends, with increasing pregnancy rates at higher numbers of follicles, although these findings were not statistically significant.

E. Influence of the Estradiol Level on Pregnancy Rate

Figure 7 presents the difference in pregnancy rates in the individual diagnostic categories as a function of estradiol level measured on the day of hCG administration. In the general population, there was only a minimal difference observed in the pregnancy rate as a function of estradiol level, with a 16.9% pregnancy rate (33 of 195 cases) for estradiol ≤ 1,000 pg/ml and an 18.0% pregnancy rate (46 of 256 cases) for estradiol > 1,000 pg/ml. In contrast, in the tubal disease group there was a significant decline in pregnancy rate at higher estradiol levels, with pregnancy rates of 28.9% (13 of 45 cases) for estradiol ≤ 1,000 pg/ml and 13.3% (8 of 60 cases) for estradiol > 1,000 pg/ml (χ^2 = 3.889, P = 0.049). The opposite trend was observed in the unexplained infertility group, where a higher pregnancy rate was seen in patients with estradiol > 1,000 pg/ml.

F. Distribution of Pregnancy Rates

Table 7 shows the distribution of pregnancy rates by diagnosis, as a function of increasing number of follicles measuring ≥ 12 mm on the day of hCG administration. The patients in each diagnostic category were stratified according to the number of follicles detected, and analyzed in increments of two follicles. The table demonstrates a wide variation in patterns between the different diagnoses, with a peak in the pregnancy rate at higher numbers of follicles for PCO, but in the middle of the range for the unexplained infertility group. The histograms shown in Figures 8A and 8B illustrate the effect of increasing numbers of follicles ≥ 12 mm on the pregnancy rate in the general and tubal disease populations. In the general population (Figure 8A), a

relatively uniform distribution is apparent, with little change in the pregnancy rate as the number of follicles increases. The tubal disease group (Figure 8B), however, demonstrates higher pregnancy rates at the lowest numbers of follicles, with rates gradually declining as the numbers of follicles increase.

G. Factors Influencing Pregnancy in Tubal Disease

The averages of variables analyzed in each cycle of IVF are presented in Table 8 for the tubal disease population, as a function of successful or unsuccessful embryo transfer. The duration of administration of hMG/FSH and the total amount of hMG/FSH used per cycle were comparable in those who achieved pregnancy and in those who did not. Mean estradiol levels in those achieving pregnancy were lower than in those who did not, although this trend was not statistically significant. The number of follicles measuring ≥ 12 mm on the day of hCG administration was significantly lower in the pregnant than in the non-pregnant patients ($P = 0.017$); a similar trend was evident between these two groups when the number of follicles measuring ≥ 16 mm on the day of hCG administration was evaluated, although this relationship was not significant. Furthermore, the number of oocytes retrieved and the number of oocytes fertilized were each lower in the pregnant than in the non-pregnant women with tubal disease. In contrast, the percentage of oocytes fertilized was higher in women who became pregnant. Although the average number of embryos transferred in the pregnant and non-pregnant groups were virtually identical, the average grade of the embryos transferred was significantly better in the pregnant group ($P = 0.007$).

Figure 9 shows the average number of transferred embryos in tubal disease patients who became pregnant, as a function of the number of follicles measuring ≥ 12 mm on the day of hCG administration. The patients in the pregnant group were stratified into two groups: those with ≤ 7 follicles measuring ≥ 12 mm and those with > 7 follicles measuring ≥ 12 mm. On average, a lower number of embryos were transferred in patients in whom a lower number of follicles were detected than in patients with higher numbers of follicles (3.65 ± 0.28 for ≤ 7 follicles vs. 4.00 ± 0.71 for > 7 follicles).

Table 9 summarizes hMG/FSH administration in tubal disease patients who subsequently became pregnant, as a function of the number of follicles measuring ≥ 12 mm in diameter on the day of hCG administration. Although the lengths of hMG/FSH administration in the stratified subgroups were approximately the same, those patients producing less follicles (≤ 7) received a slightly smaller total dose of hMG/FSH than those patients producing a larger number of follicles (> 7).

VI. DISCUSSION

The data analyzed in this study suggest that different ovarian responses to ovulation induction may affect success rates of IVF-ET in individual diagnostic groups. In particular, the study has demonstrated a strong inverse correlation between the success rate of IVF-ET in patients with tubal disease, the number of preovulatory follicles, and the level of estradiol measured on the day of hCG administration.

In this study, the number of follicles measuring ≥ 12 mm in diameter has been established as a significant predictor of IVF-ET outcome in tubal disease patients. Having identified the inverse correlation between number of follicles detected by ultrasound and pregnancy rate, a cutoff point of 7 follicles was selected for stratification, creating two tubal disease subgroups, those with ≤ 7 follicles and those with > 7 follicles. In these patients, a striking decline in pregnancy rate was observed, from 28.3% in those with ≤ 7 follicles to 8.7% in patients with > 7 follicles. Furthermore, with increasing numbers of follicles detected on ultrasound, a nearly linear descent in pregnancy rate occurred. Importantly, the higher pregnancy rate demonstrated for tubal disease patients with ≤ 7 follicles measuring ≥ 12 mm in diameter cannot be attributed to a higher number of transferred embryos. In fact, a smaller number of embryos were transferred in those patients with ≤ 7 follicles than in those with > 7 follicles. These findings extend prior observations made by Testart et al.[48] and Forman et al.[49] of an inverse relationship between the number of preovulatory follicles and IVF-ET outcome in populations combining all infertility diagnoses.

In a similar fashion, tubal disease patients in this study demonstrated a negative correlation between estradiol level measured on the day of hCG

administration and pregnancy rate. A significant decline in pregnancy rate was observed, from 28.9% in patients with estradiol levels ≤ 1,000 pg/ml to 13.3% in those with estradiol levels > 1,000 pg/ml. Although this finding is statistically significant, estradiol levels appeared to be less predictive of IVF-ET outcome than number of follicles. As such, the estradiol level on the day of hCG administration may not represent an independent predictor of IVF-ET outcome; the inverse relationship between estradiol level and pregnancy rate may simply reflect the strong positive correlation observed in this study between the number of follicles produced and the estradiol level measured.

Tubal disease patients who became pregnant were stimulated with the same average duration of gonadotropin therapy as those who did not become pregnant; despite this, those patients who became pregnant had lower estradiol levels, lower numbers of follicles detected, and lower numbers of oocytes retrieved than those who did not become pregnant. Moreover, of those patients with tubal disease who became pregnant, only slightly less gonadotropin stimulation was used in patients who produced ≤ 7 follicles than in patients with > 7 follicles. Patients with tubal disease who generated fewer follicles in response to ovulation induction produced oocytes with an increased probability of fertilization and embryos of higher quality, ultimately resulting in a greater likelihood of pregnancy.

In general, patients in the "pure" tubal disease group are expected to have little or no other pathology involved in their infertility, with presumably normal ovarian and endometrial function. Therefore, the negative correlations observed between the number of follicles, the estradiol level, and pregnancy rate may be due to overstimulation of healthy, normally tuned ovaries and endometria, producing postmature oocytes, poor quality embryos, and suboptimal implantation. This hypothesis then suggests

possibilities for changes in the ovulation induction management of patients with tubal disease, including the earlier administration of hCG. In considering this explanation, it is essential to note that no difference in gonadotropin stimulation was detected between tubal disease patients who became pregnant and those who did not, or between pregnant patients with tubal disease who produced ≤ 7 follicles or > 7 follicles. These findings highlight a rather interesting divergence in ovarian response to similar levels of stimulation, rather than an identifiable difference in the stimulation protocol between those who did or did not achieve pregnancy. As such, ovarian and folllicular appearance during gonadotropin stimulation may be more helpful in predicting IVF-ET outcomes than in defining different stimulation regimens. Prospective randomized studies assigning tubal disease patients to different ovulation induction protocols may be helpful in resolving this issue.

In addition to the findings demonstrated for tubal disease patients, the data analyzed in this study revealed trends in ovarian response and success of IVF-ET for other etiologies of infertility. Pregnancy rates for the general population correlated poorly with the number of follicles and the level of estradiol detected, contrasting with the significant inverse correlation between preovulatory follicles and IVF-ET outcome demonstrated by Forman et al.[49] for the general population. It is possible that the Forman study population, of undefined composition in the literature, may have included patients diagnosed largely with tubal disease. The study by Testart et al.[48] tended toward a decreased proportion of pregnancies with increased numbers of follicles, although this result was not statistically significant.

In contrast to patients with tubal disease, those with PCO or unexplained infertility had increased pregnancy rates with increased numbers

of follicles measuring ≥ 12 mm on the day of hCG administration. After stratifying subgroups by estradiol level, only the unexplained infertility group continued to display increased pregnancy rates with increased estradiol. In patients with a diagnosis of PCO, the endocrine manipulations inherent in ovulation induction may partially correct the underlying ovarian pathology contributing to infertility. As such, increased levels of estradiol, while generating increased numbers of follicles, may preselect those follicles of higher quality. This possibility is further substantiated by the positive correlation observed between numbers of follicles detected and percentage of oocytes fertilized. In the case of unexplained infertility, although the actual cause remains unknown, occult endometrial factors are suspected to play a pathophysiologic role; therefore, the presence of inadequate or delayed endometrial maturation in a patient with unexplained infertility might be partially corrected by controlled ovarian hyperstimulation, yielding more favorable conditions for implantation and pregnancy with higher estradiol levels. Neither the endometriosis nor the male factor groups showed distinct patterns in relation to numbers of preovulatory follicles or estradiol levels. Although the trends in IVF-ET outcome displayed by these different diagnostic groups were not statistically significant, their elucidation introduces the possibility of an optimal ovarian response, range of pretransfer parameters, and probability of success for each etiology of infertility.

A significant limitation of this study lies in the relatively small numbers of patients with "pure" diagnoses. Patients with multiple concurrent diagnoses comprised nearly one-half of the studied population. Among individual diagnostic groups, only the patients with tubal disease were well represented, with the remaining diagnostic groups numbering 28 to 36 patients each. Thus, further studies need to be performed on larger

numbers of patients in individual diagnostic categories in order to increase the power of statistical analysis. Such studies may demonstrate the optimal number of preovulatory follicles or level of estradiol more convincingly for diagnoses other than tubal disease.

The tubal disease population analyzed in this study represents only one population from one IVF center. Other populations from centers outside of this institution may be helpful in broadening the scope and applicability of the study findings.

The evaluation of egg donor cycles may allow the dissociation of ovarian and endometrial factors involved in the IVF-ET outcome. In donor cycles, only the donor ovaries are exposed to the high estradiol levels associated with ovulation induction protocols; patients receiving the embryo transfers are each given standard doses of exogenous estradiol. By controlling for post-transfer hormone levels, such studies would allow the identification of endometrial contributions to IVF-ET outcome.

Further analysis of the data may be performed with more sophisticated statistical tools. Logistic regression would allow a more precise delineation of those variables acting as independent predictors of outcome and those that have no independent predictive value.

In summary, this study has elucidated distinct patterns of ovarian response and IVF-ET outcome in individual diagnostic categories, providing exciting insight into ovarian function in controlled hyperstimulation. Two easily obtainable IVF parameters, the number of follicles measuring $\geq$ 12 mm and the level of estradiol measured on the day of hCG administration, have been identified as important predictors of outcome in patients with tubal disease and possible indicators of outcome in patients with other diagnoses. While the current practice of most IVF centers is to maximize the generation

of ovulatory size follicles in the hope of obtaining a large number of mature oocytes, the findings of this study bring into question whether this is actually beneficial for patients of specific diagnostic groups, and in particular, for patients with tubal disease. The emergence of this information necessitates the development of diagnosis-specific pretransfer parameters and individualized ovulation induction protocols in order to optimize the chances of pregnancy in patients with different etiologies of infertility.

Table 1. Distribution of the Population by Type of Cycle

Population	Age mean (range)	Number of Cycles	Number of Women
Natural Cycle	35.4 (28-42)	18	9
Egg Donor Cycle	35.6 (23-44)	5	5
Sperm Donor Cycle	38.7 (31-42)	16	10
Stimulated Cycle	34.6 (24-45)	466	184

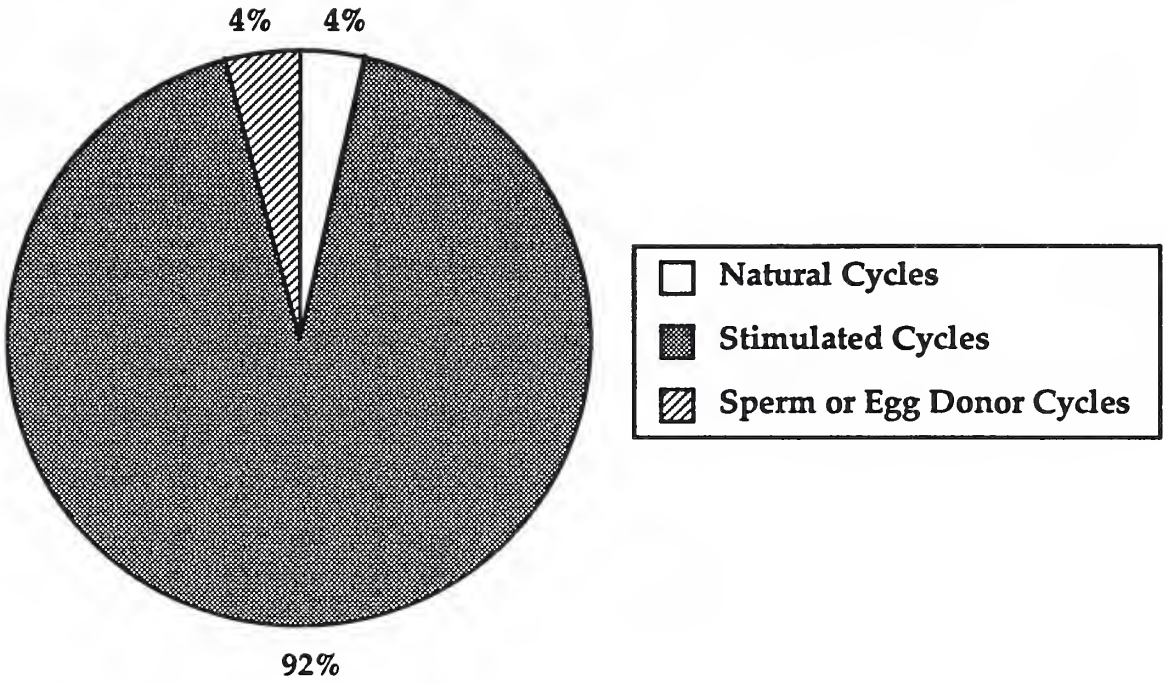

Figure 1. Distribution of the population by type of cycle (N=505 cycles).

Table 2. Distribution of the Population by Diagnosis

Population	Age mean (range)	# Cycles	# Women
General	34.6 (24-45)	466	184
Tubal Disease	33.2 (24-42)	106	45
PCO	32.0 (25-43)	34	17
Endometriosis	33.8 (29-43)	36	12
Male Factor	37.7 (29-44)	28	14
Unexplained	35.6 (30-40)	36	13

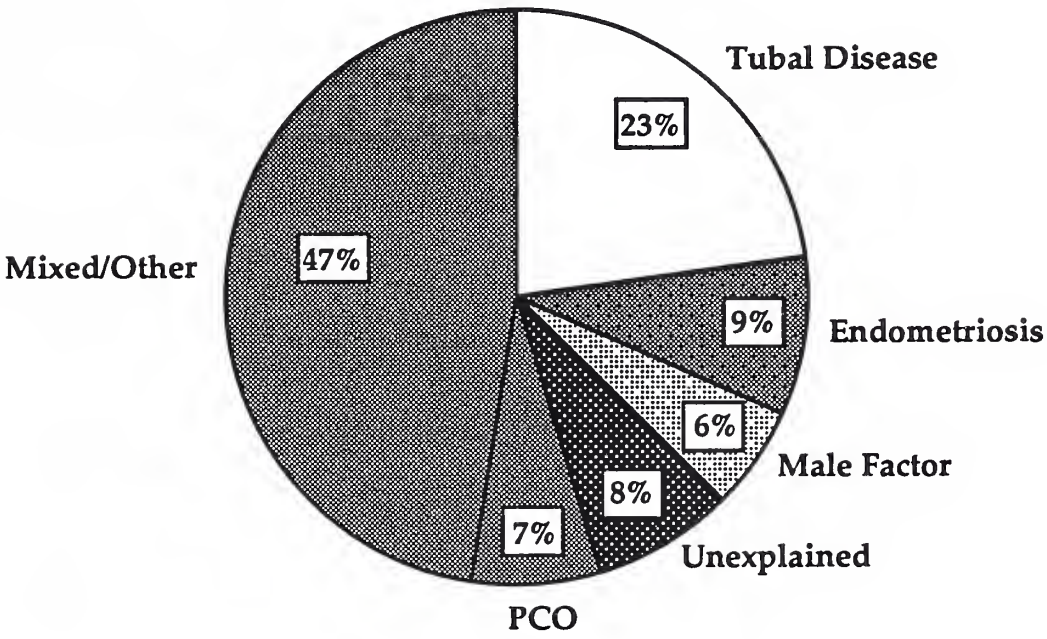

Figure 2. Distribution of the population by diagnosis.

Table 3. Distribution of Stimulation Protocols in the General Population

Stimulation Protocol	Age mean (range)	Number of Cycles	Number of Women
hMG	33.7 (24-44)	89	50
FSH	32.4 (25-39)	13	9
hMG + FSH	35.5 (28-43)	2	2
Lupron + hMG	34.8 (26-45)	238	130
Lupron + FSH	34.9 (25-44)	61	50
Lupron + hMG/FSH	33.3 (25-38)	8	6
Lupron Flare + hMG	36.9 (30-43)	19	14
Lupron Flare + FSH	38.0 (38-38)	1	1
Lupron Flare + hMG/FSH	32.7 (31-36)	3	2
Clomid	37.0 (34-40)	2	2
Clomid + hMG	33.7 (27-38)	27	11

Table 4. Parameters of IVF by Diagnosis

	Population					
Variable	General	Tubal Disease	PCO	Endometriosis	Male Factor	Unexplained
Days of hMG/FSH (#)	8.66 ± 0.12	8.82 ± 0.22	9.58 ± 0.66	8.25 ± 0.30	8.19 ± 0.38	8.38 ± 0.38
Ampoules of hMG/FSH (#)	29.12 ± 0.48	29.09 ± 0.92	21.88 ± 1.21	27.42 ± 1.33	31.65 ± 2.37	29.78 ± 1.85
Oocytes (#)	8.15 ± 0.27	8.14 ± 0.52	12.18 ± 1.50	10.17 ± 1.05	7.79 ± 1.14	7.89 ± 1.04
Oocytes Fertilized (#)	5.18 ± 0.21	5.84 ± 0.38	6.24 ± 1.23	6.11 ± 0.61	4.29 ± 0.87	5.37 ± 0.80
Oocytes Fertilized (%)	64.20 ± 1.49	74.44 ± 2.61	47.59 ± 5.19	66.14 ± 4.48	57.34 ± 6.56	69.89 ± 4.86
Embryos Transferred (#)	3.24 ± 0.09	3.72 ± 0.15	2.71 ± 0.35	4.08 ± 0.29	2.65 ± 0.38	3.29 ± 0.29
Embryos Transferred (Grade)	1.96 ± 0.04	1.86 ± 0.08	1.85 ± 0.08	2.14 ± 0.17	1.97 ± 0.19	1.96 ± 0.13

Data are presented as the mean ± SEM

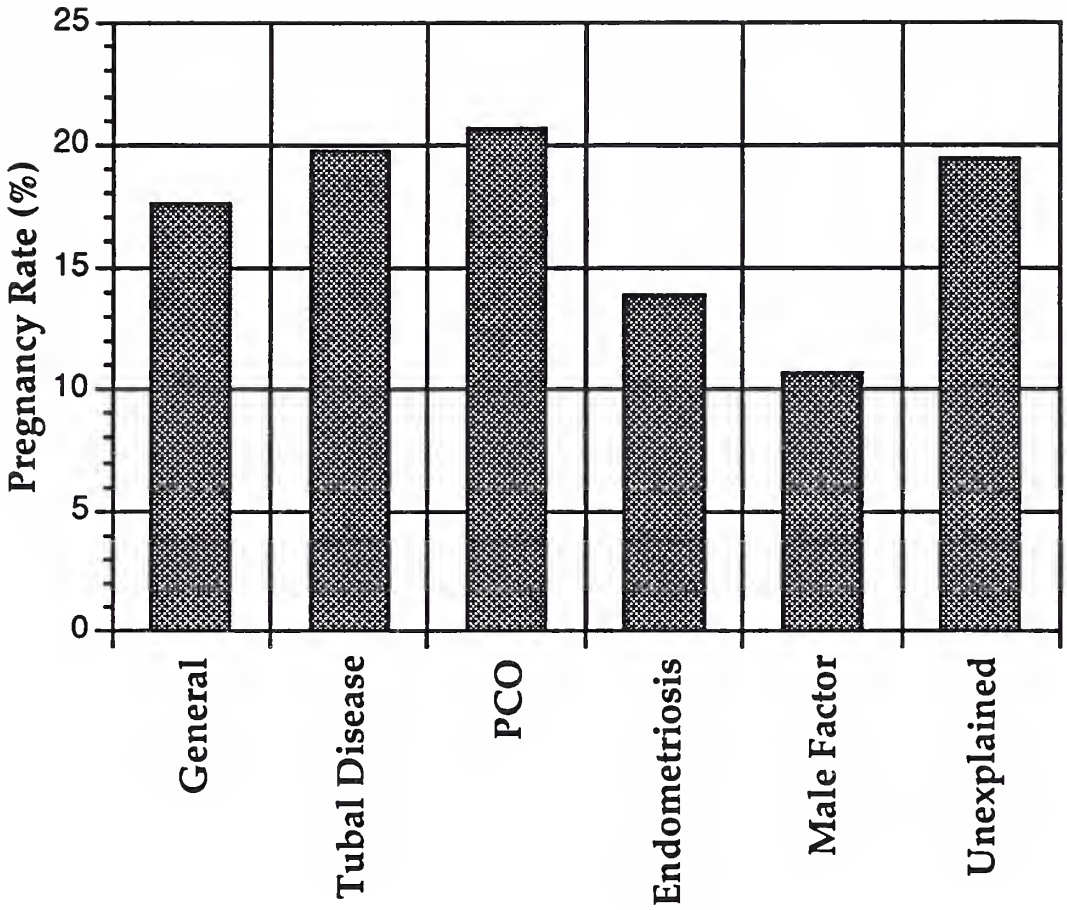

Figure 3. Pregnancy rates in individual diagnostic groups. Each bar represents the mean ± SEM.

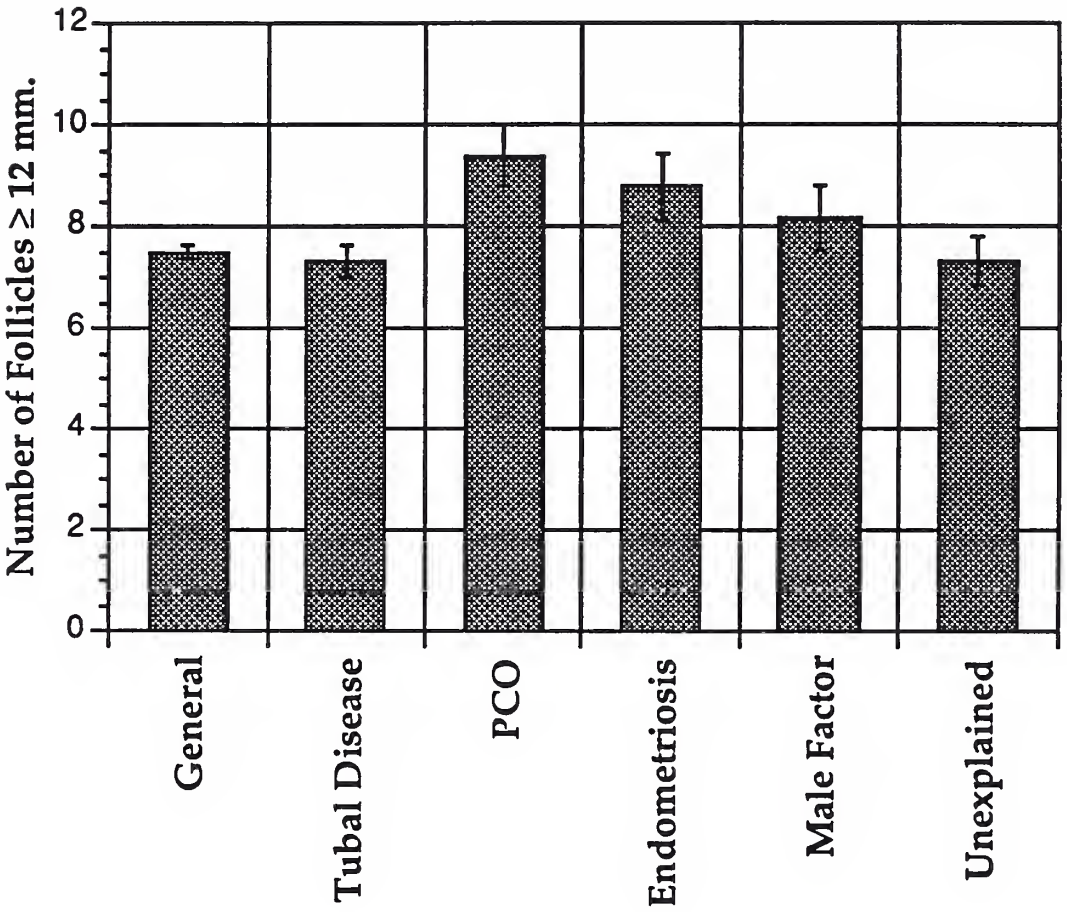

Figure 4. Number of follicles measuring ≥ 12 mm. on the day of hCG administration in individual diagnostic groups. Each bar represents the mean ± SEM.

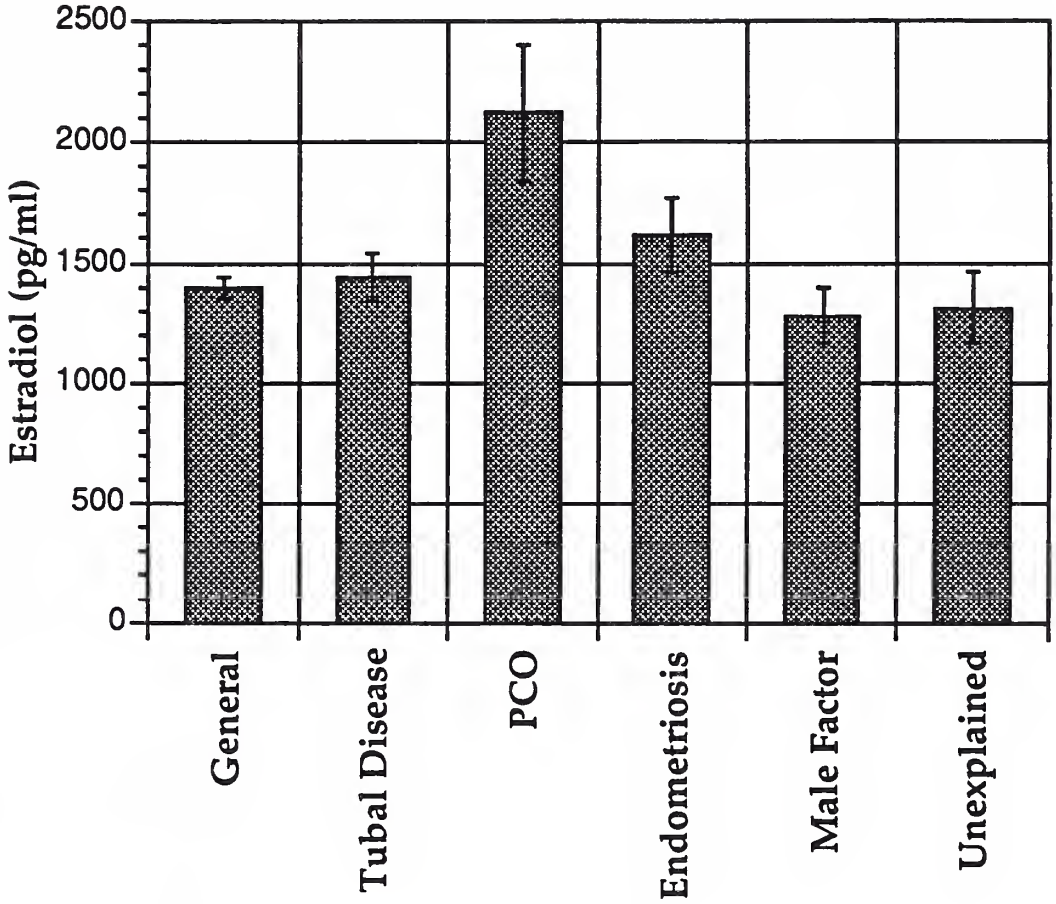

Figure 5. Estradiol levels in individual diagnostic groups. Each bar represents the mean ± SEM.

Table 5. Correlation Between the Number of Folllicles ≥ 12 mm and Other IVF Parameters

No. of Follicles ≥ 12 mm Correlated With:	Population					
	General (N=466)	Tubal Disease (N=106)	PCO (N=34)	Endometriosis (N=36)	Male Factor (N=28)	Unexplained (N=36)
Days of hMG/FSH (#)	r = 0.056^{NS}	r = 0.034^{NS}	r = -0.117^{NS}	r = 0.205^{NS}	r = 0.137^{NS}	r = 0.092^{NS}
Ampoules of hMG/FSH (#)	r = -0.050^{NS}	r = -0.105^{NS}	r = 0.198^{NS}	r = 0.096^{NS}	r = 0.060^{NS}	r = -0.174^{NS}
Estradiol	r = 0.572***	r = 0.669***	r = 0.395*	r = 0.458**	r = 0.401^{NS}	r = 0.688***
Oocytes (#)	r = 0.576***	r = 0.503***	r = 0.452**	r = 0.441**	r = 0.552**	r = 0.661***
Oocytes Fertilized (#)	r = 0.400***	r = 0.395***	r = 0.138^{NS}	r = 0.513*	r = 0.413*	r = 0.410*
Oocytes Fertilized (%)	r = -0.093*	r = -0.217*	r = 0.035^{NS}	r = -0.105^{NS}	r = 0.117^{NS}	r = -0.188^{NS}
Embryos Transferred (#)	r = 0.274***	r = 0.247*	r = 0.149^{NS}	r = 0.254^{NS}	r = 0.213^{NS}	r = 0.112^{NS}
Embryos Transferred (Grade)	r = -0.065^{NS}	r = 0.113^{NS}	r = 0.002^{NS}	r = -0.485^{NS}	r = -0.296^{NS}	r = -0.163^{NS}

* P < 0.05; ** P < 0.01; *** P < 0.001; NS = not significant

Table 6. Correlation Between the Number of Oocytes Retrieved and Other IVF Parameters

Number of Oocytes Retrieved Correlated With:	Population					
	General (N=466)	Tubal Disease (N=106)	PCO (N=34)	Endometriosis (N=36)	Male Factor (N=28)	Unexplained (N=36)
Days of hMG/FSH (#)	$r = -0.054^{NS}$	$r = -0.137^{NS}$	$r = -0.233^{NS}$	$r = -0.293^{NS}$	$r = -0.340^{NS}$	$r = 0.099^{NS}$
Ampoules of hMG/FSH (#)	$r = -0.121^{**}$	$r = -0.119^{NS}$	$r = 0.275^{NS}$	$r = -0.223^{NS}$	$r = -0.437^{*}$	$r = -0.086^{NS}$
Estradiol	$r = 0.582^{***}$	$r = 0.543^{***}$	$r = 0.026^{***}$	$r = 0.624^{***}$	$r = 0.602^{**}$	$r = 0.694^{***}$
Oocytes Fertilized (#)	$r = 0.774^{***}$	$r = 0.821^{***}$	$r = 0.734^{***}$	$r = 0.698^{***}$	$r = 0.773^{***}$	$r = 0.767^{***}$
Oocytes Fertilized (%)	$r = -0.049^{NS}$	$r = -0.221^{*}$	$r = 0.212^{NS}$	$r = -0.372^{*}$	$r = -0.089^{NS}$	$r = -0.126^{NS}$
Embryos Transferred (#)	$r = 0.456^{***}$	$r = 0.517^{***}$	$r = 0.221^{NS}$	$r = 0.378^{*}$	$r = 0.358^{NS}$	$r = 0.455^{**}$
Embryos Transferred (Grade)	$r = -0.145^{*}$	$r = 0.139^{NS}$	$r = 0.188^{NS}$	$r = -0.575^{*}$	$r = -0.485^{NS}$	$r = -0.028^{NS}$

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS = not significant

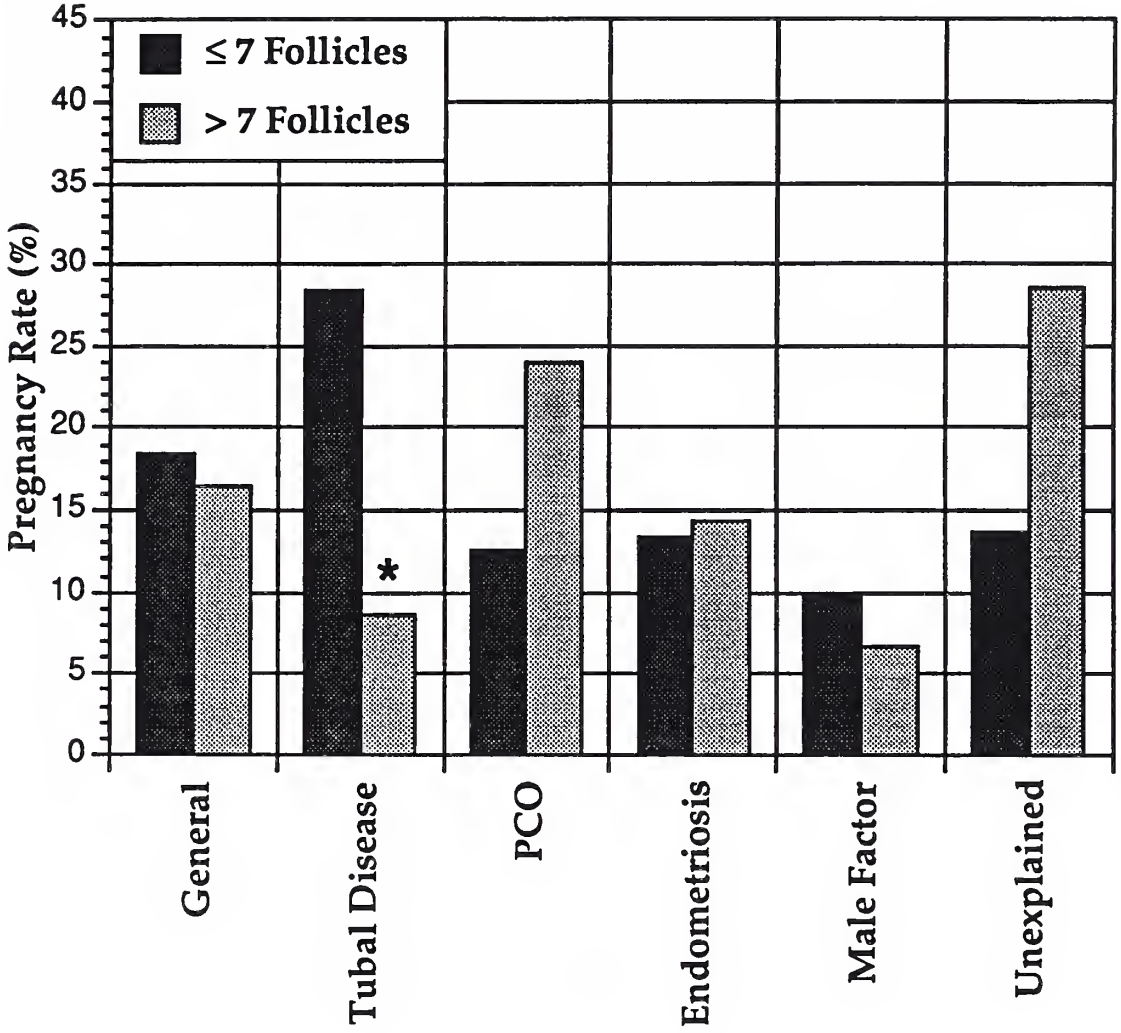

Figure 6. Pregnancy rate by diagnosis as a function of the number of follicles measuring ≥ 12 mm on the day of hCG administration. Patients in all diagnostic categories were divided into two subgroups based upon the number of follicles detected. Each bar represents the mean ± SEM. The asterisk denotes a significant difference in the means for Tubal Disease (P = 0.014)

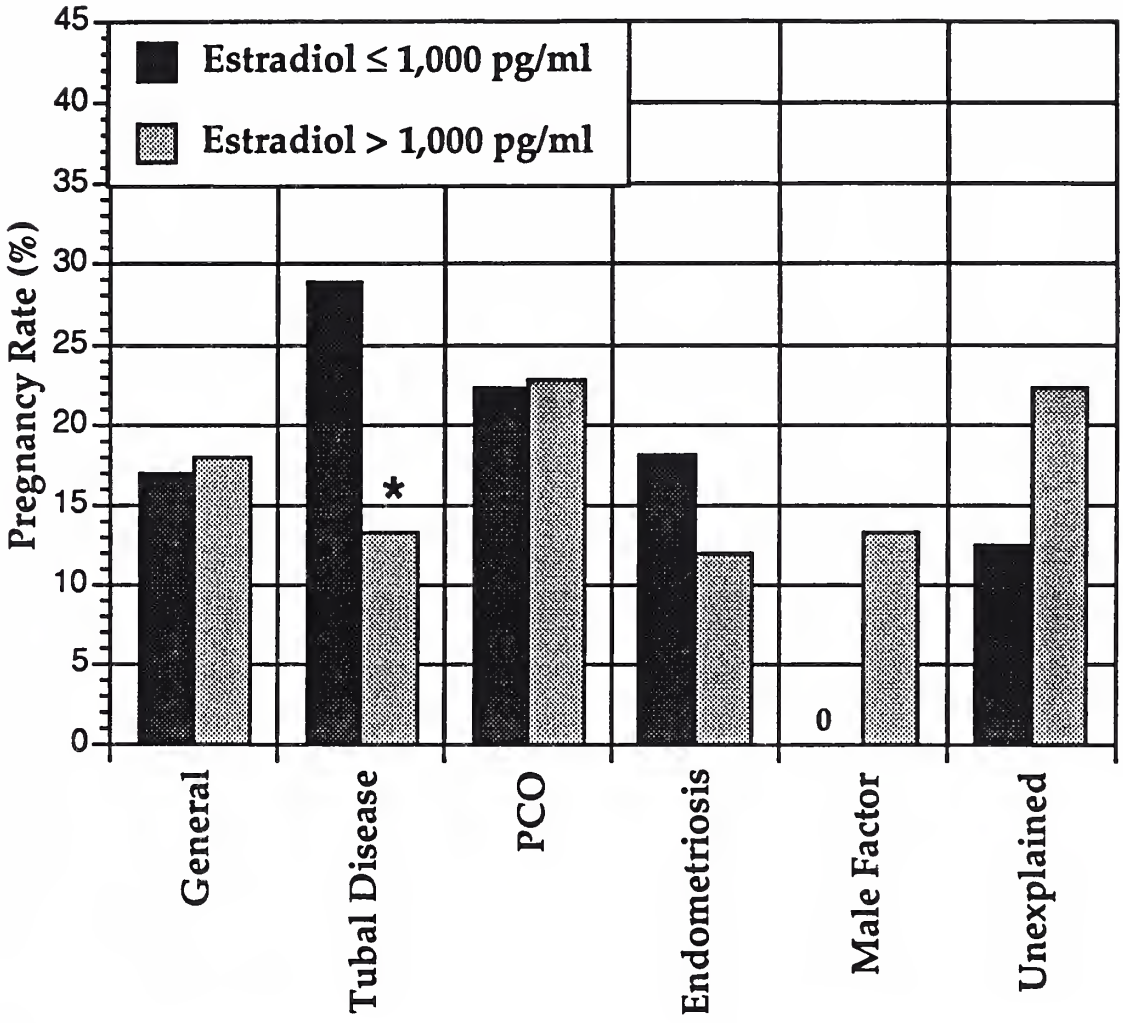

Figure 7. Pregnancy rate by diagnosis as a function of the estradiol level measured on the day of hCG administration. All diagnostic categories were divided into two subgroups based upon the estradiol level measured. Each bar represents the mean ± SEM. The asterisk denotes a significant difference in the means for Tubal Disease (P = 0.049)

Table 7. Numbers and Rates of Pregnancies per Number of Follicles ≥ 12 mm

Number of Follicles	Population											
	General		Tubal Disease		PCO		Endometriosis		Male Factor		Unexplained	
	%	N	%	N	%	N	%	N	%	N	%	N
1 - 2	7.7	13	50.0	2	-	0	0	1	-	0	0	0
3 - 4	18.8	80	33.3	21	25.0	4	20.0	5	0	3	0	7
5 - 6	15.8	114	20.8	24	0	4	11.1	9	0	5	0	9
7 - 8	20.6	97	13.8	29	33.3	3	0	1	12.5	8	37.5	8
9 - 10	19.1	68	10.0	10	33.3	9	25.0	8	0	3	42.9	7
11 - 12	19.6	51	30.0	10	12.5	8	20.0	5	25.0	4	25.0	4
13 - 14	8.3	24	0	8	0	3	0	4	0	1	-	0
15 - 16	14.3	14	0	2	50.0	2	0	3	0	1	0	1
≥ 17	0	1	-	0	-	0	-	0	-	0	-	0

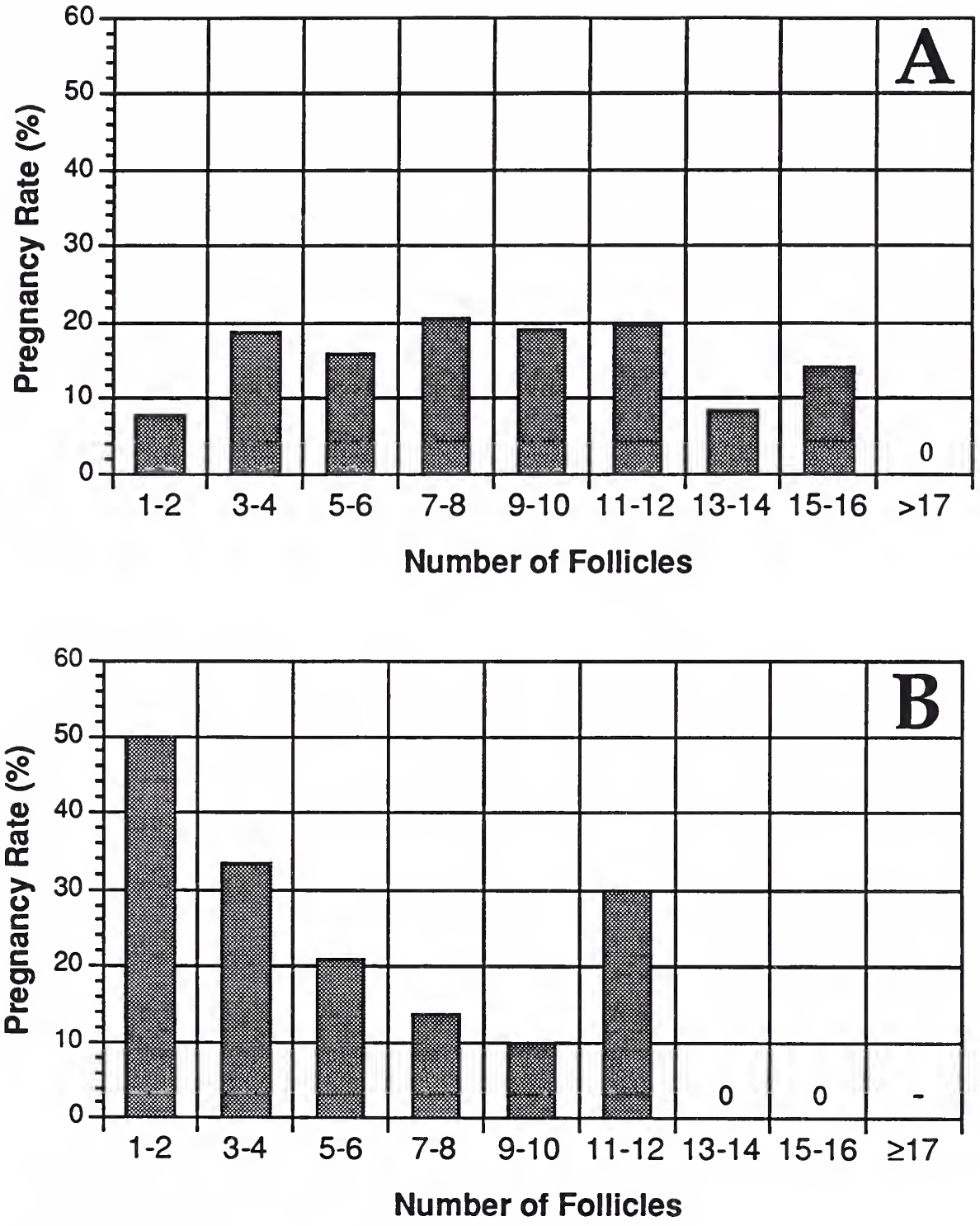

Figure 8. Effect of the number of follicles ≥ 12 mm. on the day of hCG administration on the pregnancy rate in (A) the General population (N=466) and (B) the Tubal Disease population (N=106).

Table 8. IVF Parameters by Pregnancy Outcome for Tubal Disease Population

	Pregnancy	
Variable	**Yes**	**No**
Age	33.4 ± 0.9	33.1 ± 0.6
Days of hMG/FSH (#)	8.7 ± 0.5	8.8 ± 0.2
Ampoules of hMG/FSH (#)	29.6 ± 2.3	29.0 ± 1.0
Estradiol	1233 ± 217	1492 ± 114
Follicles ≥ 12 mm (#)	5.9 ± 0.6*	7.7 ± 0.3
Follicles ≥16 mm (#)	2.6 ± 0.4	3.4 ± 0.2
Oocytes (#)	7.0 ± 0.9	8.4 ± 0.6
Oocytes Fertilized (#)	5.1 ± 0.5	6.0 ± 0.5
Oocytes Fertilized (%)	80.1 ± 4.1	73.0 ± 3.1
Embryos Transferred (#)	3.7 ± 0.3	3.7 ± 0.2
Embryos Transferred (Grade)	1.5 ± 0.1**	2.0 ± 0.1

* $p = 0.017$
** $p = 0.007$

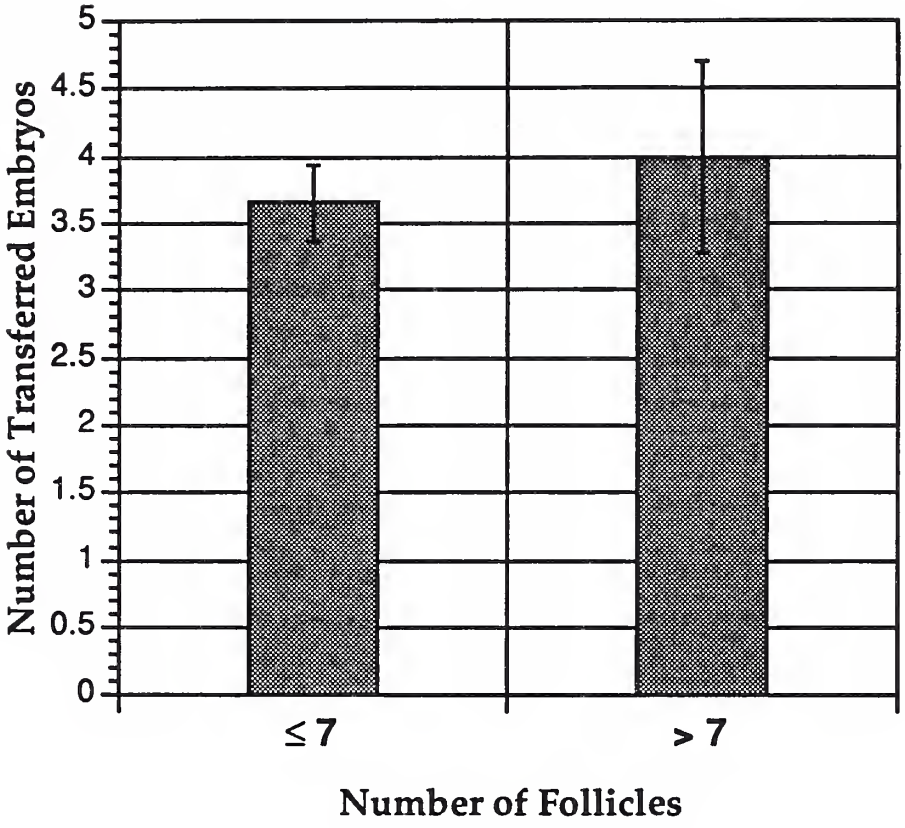

Figure 9. Number of transferred embryos in pregnant patients with Tubal Disease, as a function of the number of follicles measuring ≥ 12 mm in diameter on the day of hCG administration. Each bar represents the mean ± SEM.

Table 9. hMG/FSH Administration in Pregnant Tubal Disease Patients

	Number of Follicles ≥ 12 mm	
Variable	**≤ 7**	**> 7**
Days of hMG/FSH (#)	29.5 ± 2.8	30.0 ± 3.5
Ampoules of hMG/FSH (#)	8.6 ± 0.5	9.3 ± 0.9

Data are presented as the mean ± SEM.

VIII. REFERENCES

1 Menning BE. The psychology of infertility. In: Aiman J, ed. Infertility: diagnosis and management. New York, Springer-Verlag, 1984: 17-29.

2 Bradshaw KD, Carr BR. Modern diagnostic evaluation of the infertile couple. In: Carr BR, ed. Textbook of reproductive medicine. Norwalk, Appleton & Lange, 1993: 443-452.

3 Mishell DRJ. Infertility. In: Manning S, ed. Comprehensive gynecology. 2nd ed. St. Louis, Mosby-Year Book, Inc., 1992: 1189-1244.

4 Schlaff WD, Hassiakos DK, Damewood MD, Rock JA. Neosalpingostomy for distal tubal obstruction: prognostic factors and impact of surgical technique. Fertil Steril 1990;54:984-990.

5 Droegemueller W. Endometriosis and adenomyosis. In: Manning S, ed. Comprehensive gynecology. 2nd ed. St. Louis, Mosby-Year Book, Inc., 1992: 545-576.

6 Olive DL, Stohs GF, Metzger DA, Franklin RR. Expectant management and hydrotubations in the treatment of endometriosis-associated infertility. Fertil Steril 1985;44:35-41.

7 Seibel MM. Ovarian Dysfunction and anovulation. In: Carr BR, ed. Textbook of reproductive medicine. Norwalk, Appleton & Lange, 1993: 355-369.

8 Quigley ME, Rakoff JS, Yen SSC. Increased luteinizing sensitivity to dopamine inhibition in polycystic ovary syndrome. J Clin Endocrinol Metab 1981;52:231-234.

9 Falaschi P, Rocco A, Del Pozo E. Inhibitory effect of bromocriptine treatment on luteinizing hormone secretion in polycystic ovary syndrome. J Clin Endocrinol Metab 1986;62:348-351.

10 Erickson GF, Hsueh AJW, Quigley ME, Rebar RW, Yen SSC. Functional studies of aromatase activity in human granulosa cells from normal and polycystic ovaries. J Clin Endocrinol Metab 1979;49:514-519.

11 Chang RJ, Mandel FP, Wolfsen AR, Judd HL. Circulating levels of plasma adrenocorticotropin in polycystic ovary disease. J Clin Endocrinol Metab 1982;54:1265-1267.

12 Cramer OM, Parker CR, Porter JC. Estrogen inhibition of dopamine release into hypophyseal portal blood. Endocrinology 1979;104:419-422.

13 McConnell JD. Diagnosis and treatment of male infertility. In: Carr BR, ed. Textbook of reproductive medicine. Norwalk, Appleton & Lange, 1993: 453-468.

14 MacLeod J. Human male infertility. Obstetrical and Gynecological Survey 1971;26:335-351.

15 Simmons FA. Human infertility. New England Journal of Medicine 1956;255:1140-1146.

16 Davis OK, Rosenwaks Z. Assisted reproductive technology. In: Carr BR, ed. Textbook of reproductive medicine. Norwalk, Appleton & Lange, 1993: 571-586.

17 Honea KL. Understanding unexplained infertility. In: Carr BR, ed. Textbook of reproductive medicine. Norwalk, Appleton & Lange, 1993: 537-545.

18 Barnea ER, Holford TR, McInnes DRA. Long-term prognosis of infertile couples with normal basic investigations: a life-table analysis. Obstetrics and Gynecology 1985;66:24-26.

19 Navot D, Rosenwaks Z, Margalioth EJ. Prognostic assessment of female fecundity. Lancet 1987;2:645-647.

20 Steptoe PC, Edwards RG, Walters DE. Observations on 767 clinical pregnancies and 500 births after human *in vitro* fertilization. Hum Reprod 1986;1:89-94.

21 Edwards RG. *In vitro* fertilization and embryo replacement. Annals of the N.Y. Academy of Sciences 1985;442:1-22.

22 Hodgen GD. Ovarian function for multiple follicle maturation. Clinical Obstetrics and Gynecology 1986;29:127-140.

23 Mishell DRJ. Reproductive endocrinology. In: Manning S, ed. Comprehensive gynecology. 2nd ed. St. Louis, Mosby-Year Book, Inc., 1992: 79-142.

24 Hirshfield AN. Development of follicles in the mammalian ovary. International Review of Cytology 1991;124:43-100.

25 Peters H. The development of the mouse ovary from birth to maturity. Acta Endocrinol 1969;62:98-116.

26 Schwartz NB. The role of FSH and LH and of their antibodies on follicle growth and on ovulation. Biology of Reproduction 1974;10:236-272.

27 Macnamee MC, Brinsden PR. Superovulation strategies in assisted conception. In: Brinsden PR and Rainsbury PA, eds. A textbook of in vitro fertilization and assisted reproduction. Park Ridge, The Parthenon Publishing Group Inc., 1992: 111-125.

28 Dekel N. Spatial relationship of follicular cells in the control of meiosis. In: Haseltine FB, First NI, eds. Progress in clinical and biological research. New York, Alan R. Liss, Inc., 1988: 87-151.

29 Eppig JJ, Downs SM. The effect of hypoxanthine on mouse oocyte growth and development *in vitro*: maintenance of meiotic arrest and gonadotropin-induced oocyte maturation. Developmental Biology 1987;119:313-321.

30 Stanger JD, Yovich JL. Reduced *in-vitro* fertilization of human oocytes from patients with raised basal luteinizing hormone levels during the follicular phase. British Journal of Obstetrics and Gynaecology 1985;92:385-393.

31 Howles CM, MacNamee MC, Edwards RG, Goswamy R, Steptoe PC. Effect of high tonic levels of luteinising hormone on outcome of *in vitro* fertilisation. The Lancet 1986;2:521-522.

32 Thomas A, Okamoto S, O'Shea F, Maclachlan V, Besanko M, Healy D. Do raised serum luteinizing hormone levels during stimulation for *in-vitro* fertilization predict outcome? British Journal of Obstetrics and Gynaecology 1989;96:1328-1332.

33 Fleming R, Coutts JRT. Induction of multiple follicular development for IVF. British Medical Bulletin 1990;46:596-615.

34 Hughes EG, King C, Wood EC. A prospective study of prognostic factors in in vitro fertilization and embryo transfer. Fertil Steril 1989;51:838-844.

35 Romeu A, Muasher SJ, Acosta AA, et al. Results of in vitro fertilization attempts in women 40 years of age and older: the Norfolk experience. Fertil Steril 1987;47:130-136.

36 Liu DY, Du Plessis YP, Nayudu PL, Johnston WIH, Baker HWG. The use of in vitro fertilization to evaluate putative tests of human sperm function. Fertil Steril 1988;49:272-277.

37 Mahadevan MM, Trounson AO. The influence of seminal characteristics on the success rate of human in vitro fertilization. Fertil Steril 1984;42:400-405.

38 Puissant F, Van Rysselberge M, Barlowe P, Deweze J, Leroy F. Embryo scoring as a prognostic tool in IVF treatment. Hum Reprod 1987;2:705-708.

39 Jones HWJ, Acosta A, Andrews MC, et al. The importance of the follicular phase to success and failure in in vitro fertilization. Fertil Steril 1983;40:317-321.

40 Dor J, Rudak E, Mashiach S, Nebel L, Serr D, Goldman B. Periovulatory 17ß-estradiol changes and embryo morphologic features in conception and nonconceptional cycles after human in vitro fertilization. Fertil Steril 1986;45:63-68.

41 Howles CM, Macnamee MC, Edwards RG. Follicular development and early luteal function of conception and non-conceptional cycles after human in-vitro fertilization: endocrine correlates. Hum Reprod 1987;2:17-21.

42 Zenzes MT, Belkien L, Bordt J, Kan I, Schneider HPG, Nieschlag E. Cytologic investigation of human in vitro fertilization failures. Fertil Steril 1985;43:883-891.

43 Olivennes F, Aymar C, Bomsel-Helmreich O, Frydman R. Oocyte quality and collection: assessment of oocyte quality in an IVF program. Assisted Reproduction Reviews 1993;3:218-223.

44 Bomsel-Helmreich O, Huyen LVN, Durand-Gasselin I, Salat-Baroux J, Antoine JM. Mature and immature oocytes in large and medium follicles after clomiphene citrate and human menopausal gonadotropin stimulation without human chorionic gonadotropin. Fertil Steril 1987;48:596-604.

45 Pellicer A, Ruiz A, Castellvi RM, et al. Is the retrieval of high numbers of oocytes desirable in patients treated with gonadotrophin-releasing hormone analogues (GnRHa) and gonadotrophins? Hum Reprod 1989;4:536-540.

46 Testart J, Belaisch-Allart J, Forman R, et al. Influence of different stimulation treatments on oocyte characteristics and in-vitro fertilizing ability. Hum Reprod 1989;4:192-197.

47 Sharma V, Riddle A, Mason BA, Pampiglione J, Campbell S. An analysis of factors influencing the establishment of a clinical pregnancy in an ultrasound-based ambulatory in vitro fertilization program. Fertil Steril 1988;49:468-478.

48 Testart J, Belaisch-Allart J, Frydman R. Relationships between embryo transfer results and ovarian response and in vitro fertilization rate: analysis of 186 human pregnancies. Fertil Steril 1986;45:237-243.

49 Forman RG, Robinson J, Egan D, Ross C, Gosden B, Barlow DH. Follicular monitoring and outcome of in vitro fertilization in gonadotropin-releasing hormone-agonist-treated cycles. Fertil Steril 1991;5:567-573.

50 Andrews WC, Buttrom VC, Behrman SJea. Revised American Fertility Society classification of endometriosis: 1985. Fertil Steril 1985;43:351-352.

51 Huszar G, Vigue L. Spermatogenesis-related change in the synthesis of the creatinine kinase B-type and M-type isoforms in human spermatozoa. Molecular Reproduction and Development 1990;25:258-262.

52 Huszar G, Vigue L, Morshedi M. Sperm creatinine phosphokinase M-isoform ratios and fertilizing potential of men: a blinded study of 84 couples treated with in vitro fertilization. Fertil Steril 1992;57:882-888.